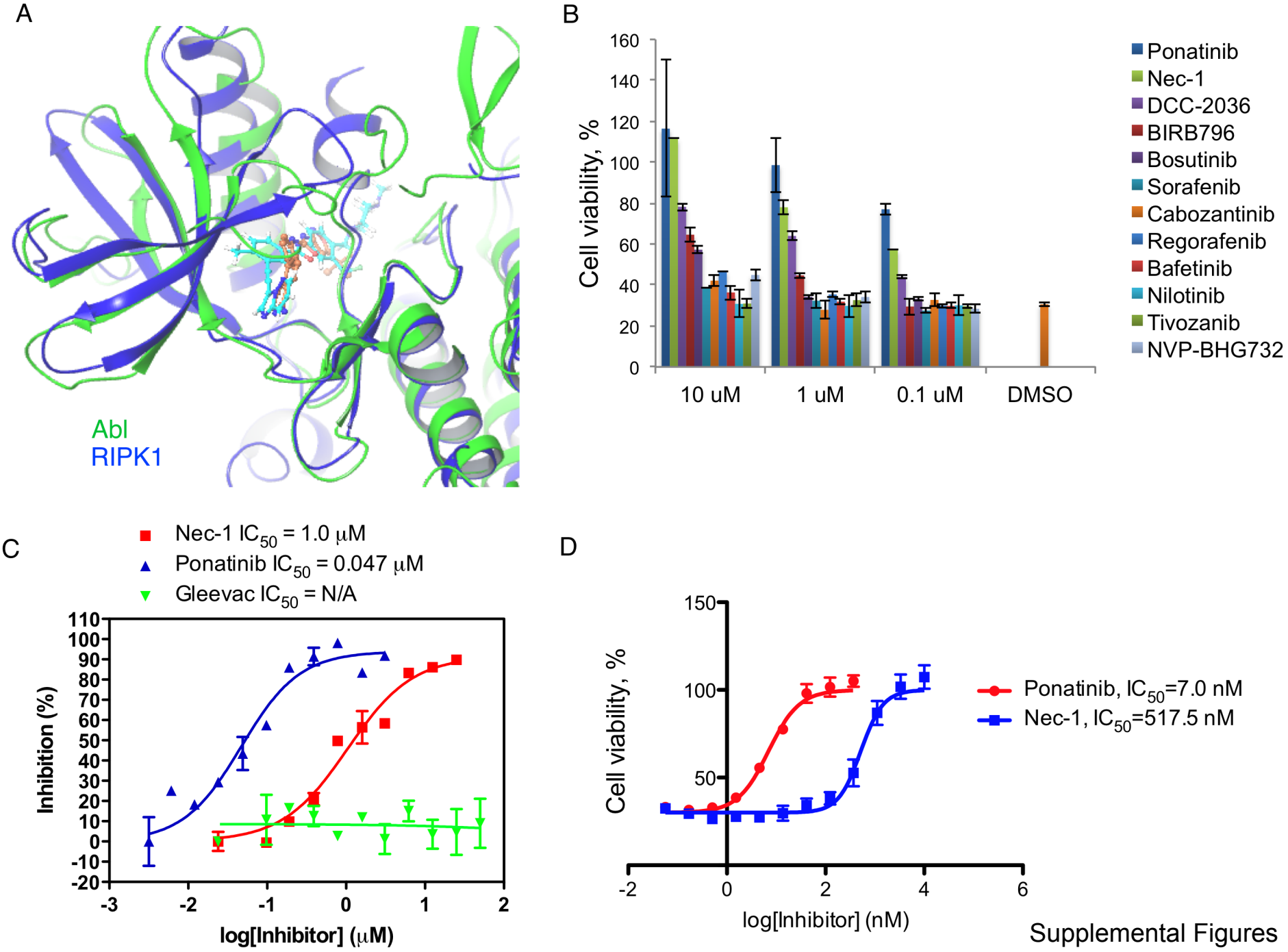
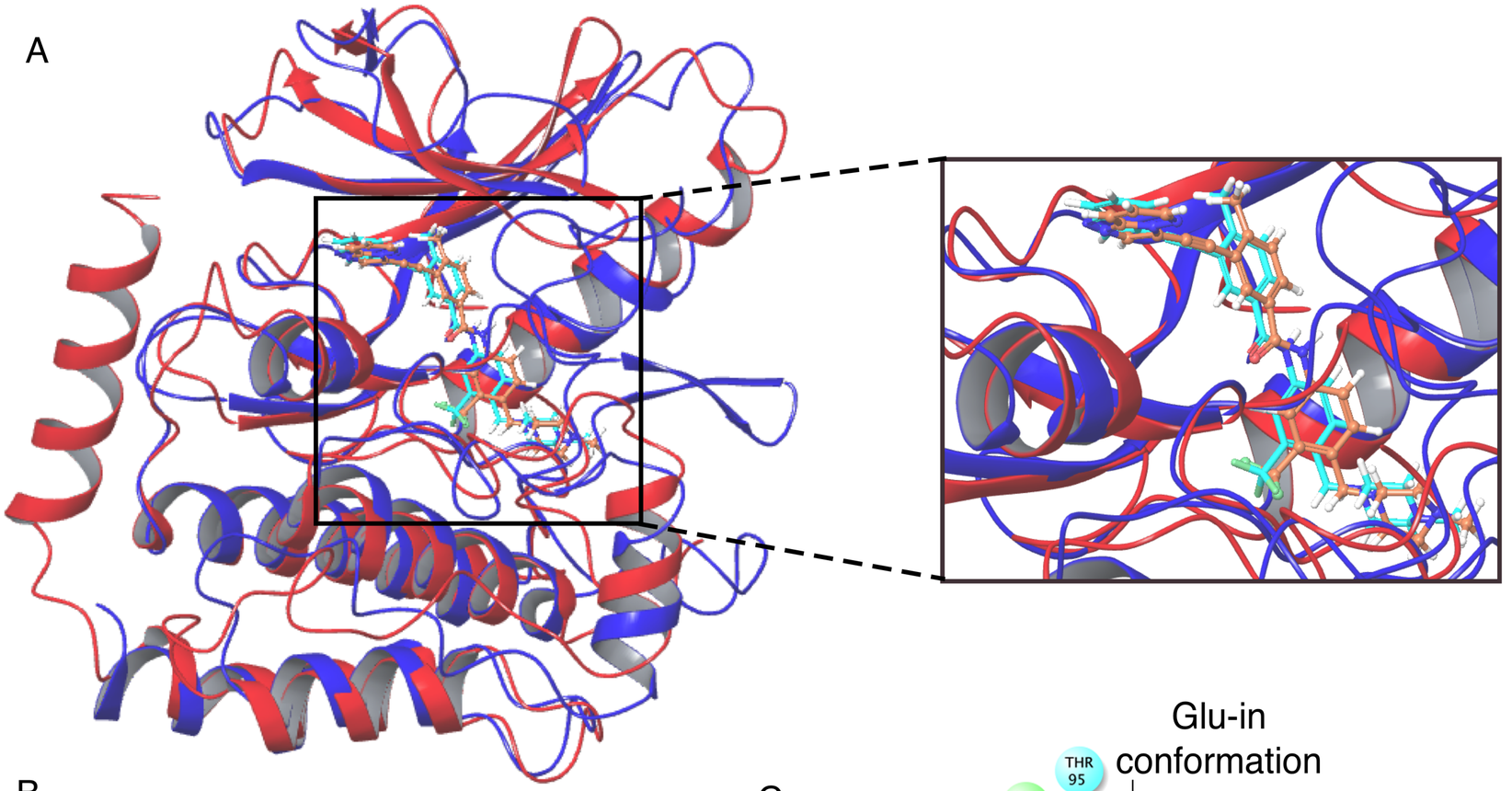


Supplemental Figures
Figure S1

A



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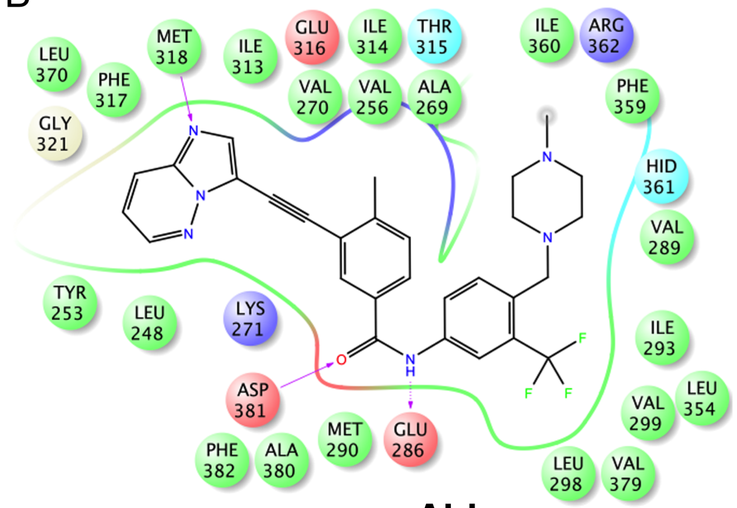
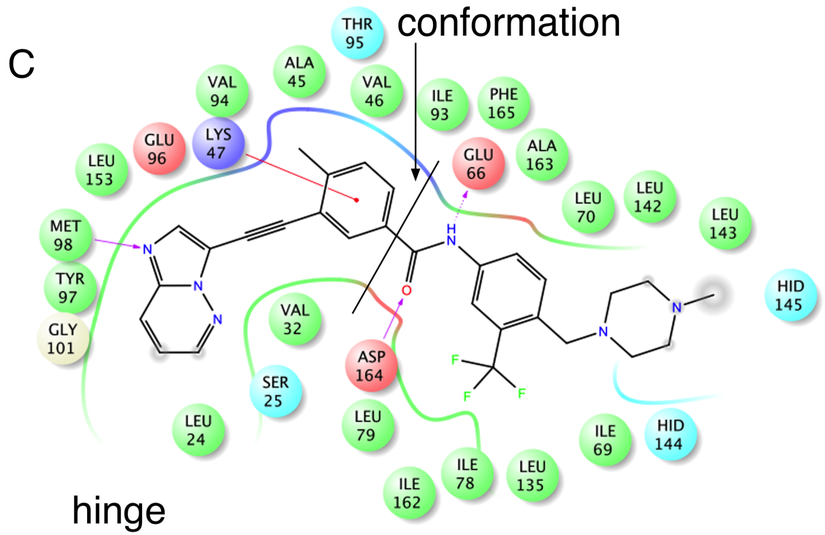


Abl

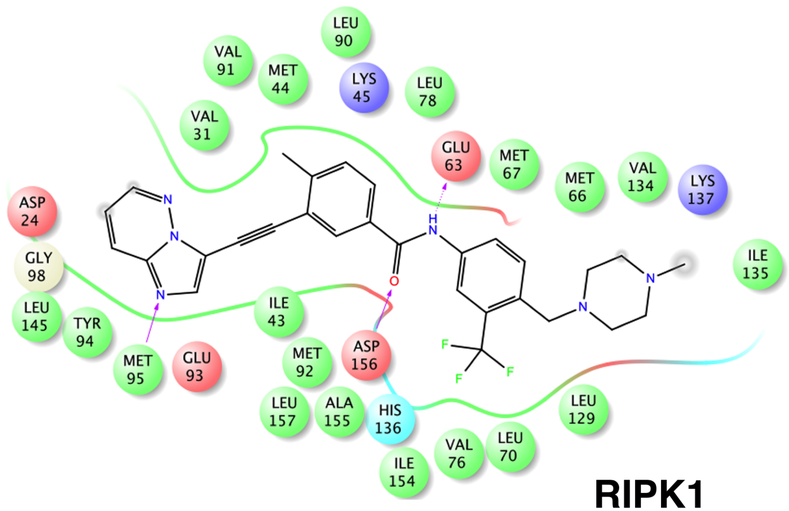
C



RIPK2

DFG-out pocket

D

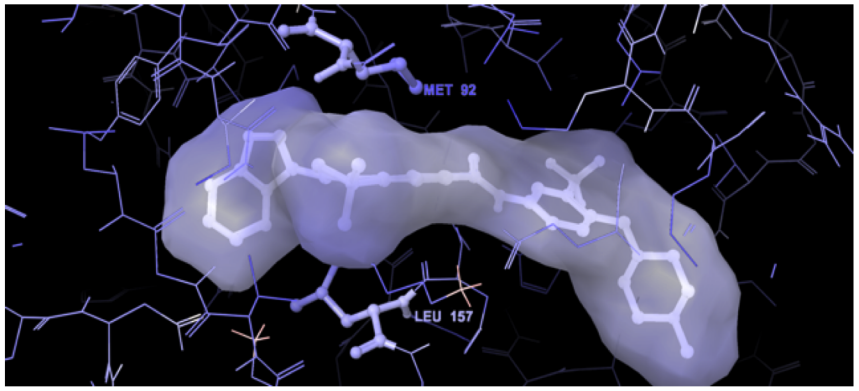


RIPK1

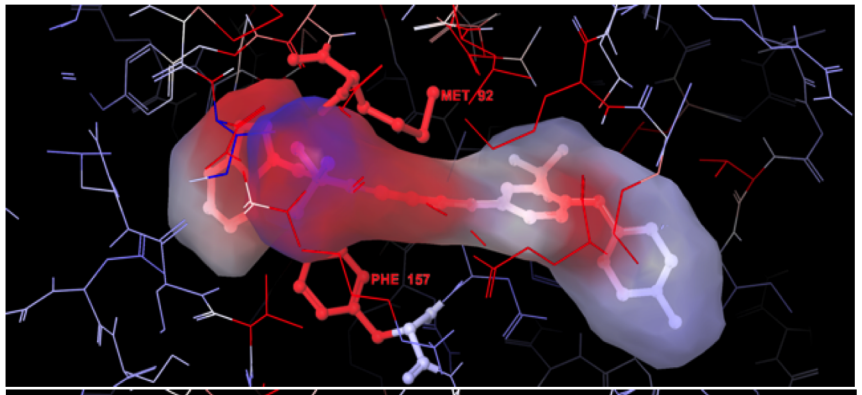
Figure S2

A

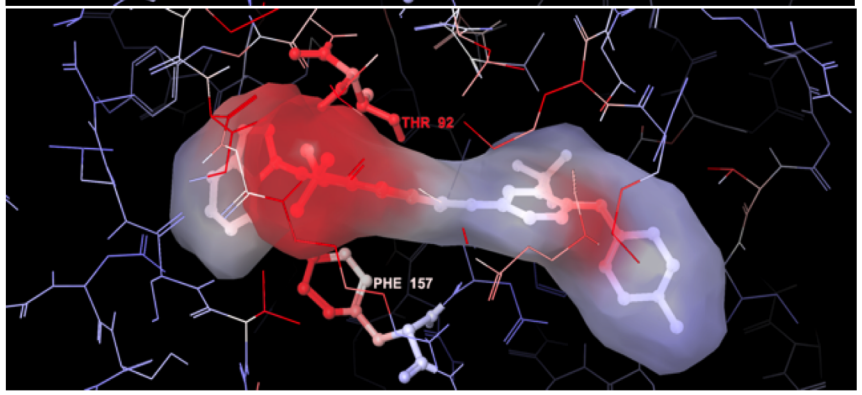
RIPK1 - ponatinib



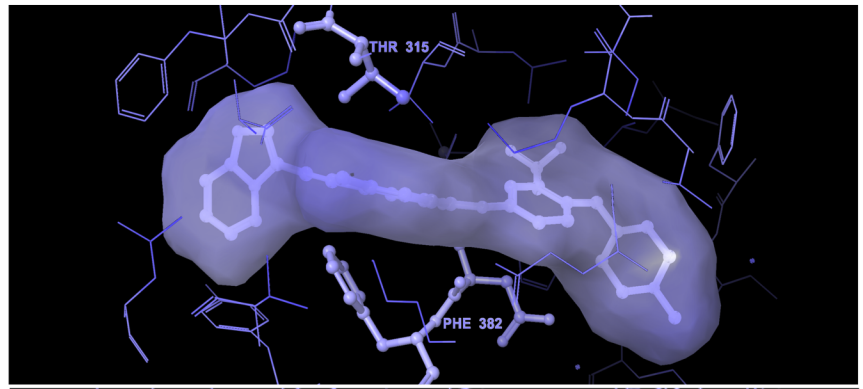
F157L RIPK1 - CS6



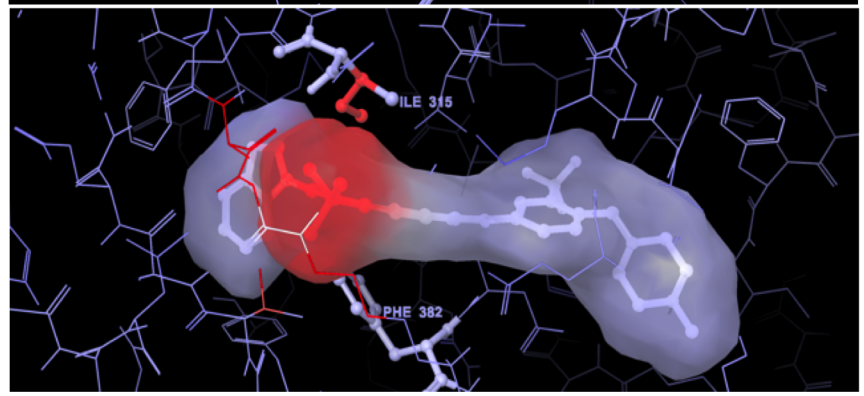
F157L/T92M RIPK1 - CS6



Abl - ponatinib



T315I Abl - CS6



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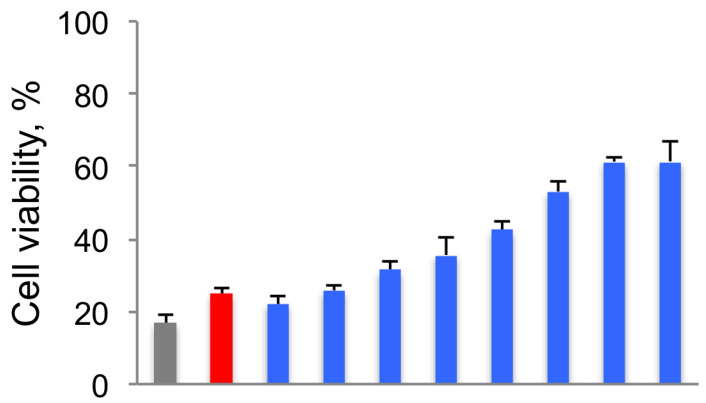
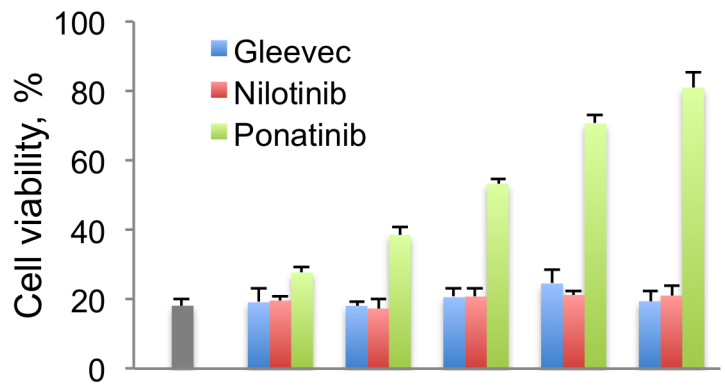


IFN	+	+	+	+	+	+	+	+	+	+
Nec-1	-	+	-	-	-	-	-	-	-	-
GSK-872, μM	-	-	0.01	0.1	0.25	0.5	1	2.5	5	10

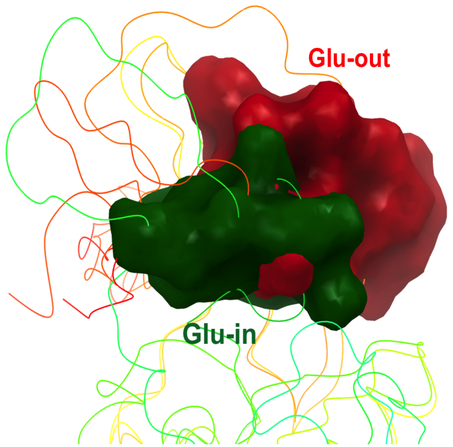
C



Nilotinib/ Gleevec, μM	-	0.5	1	2.5	5	10
Ponatinib, nM	-	10	100	250	500	1000

Figure S3

A



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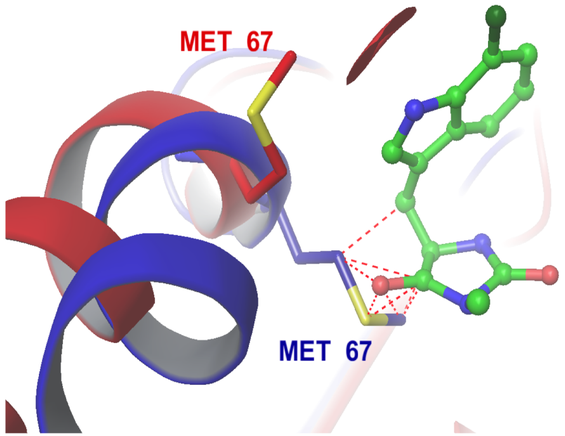

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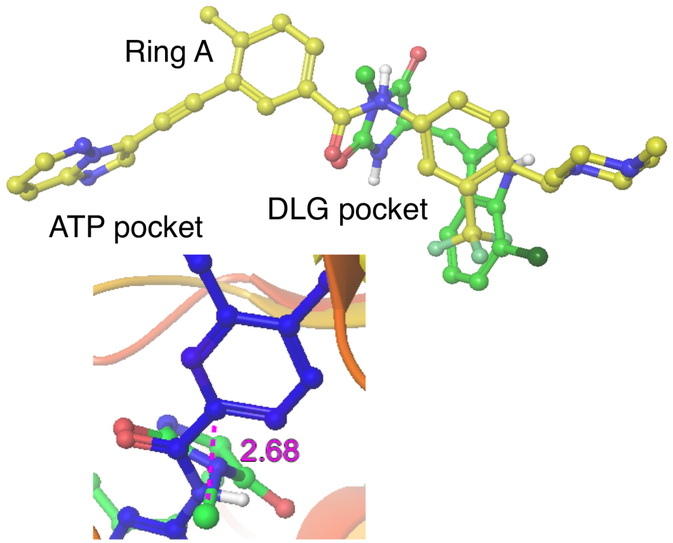

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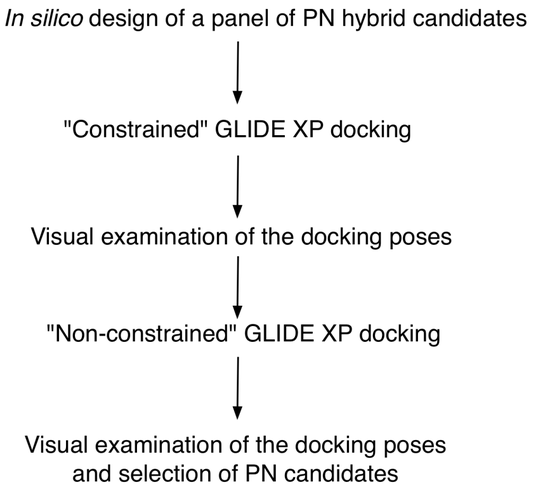

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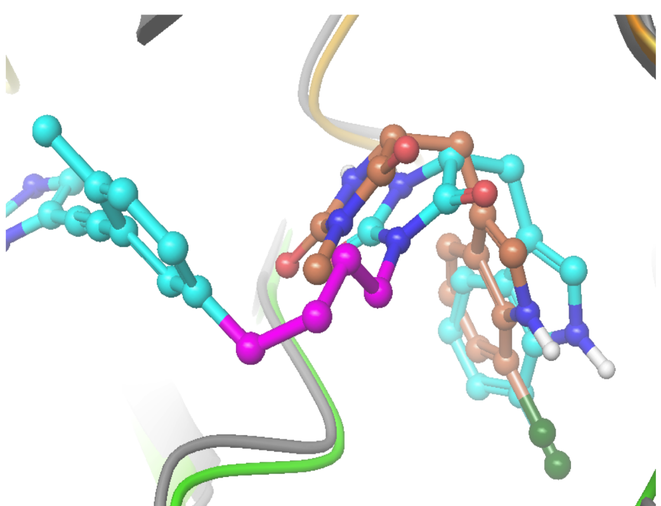
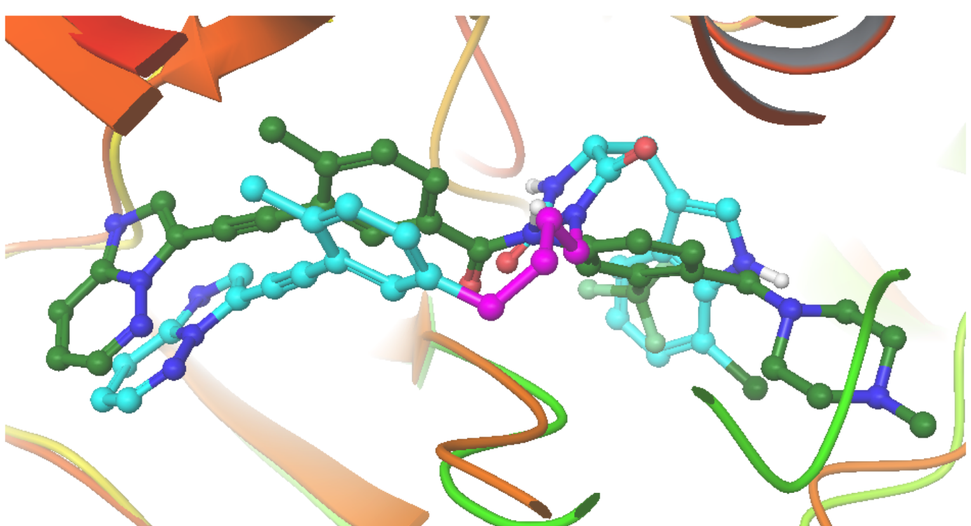


Nec-1/PN10



Ponatinib/PN10

F

	Constrained GLIDE XP docking, score	Non-constrained GLIDE XP docking, score
PN7	-13.491	-13.298
PN8	-15.266	-13.268
PN9	-15.228	-14.981
PN10	-16.961	-15.313
PN11	-11.54	-13.003
PN12	ND	-10.752
PN13	-14.916	-15.114

Figure S4

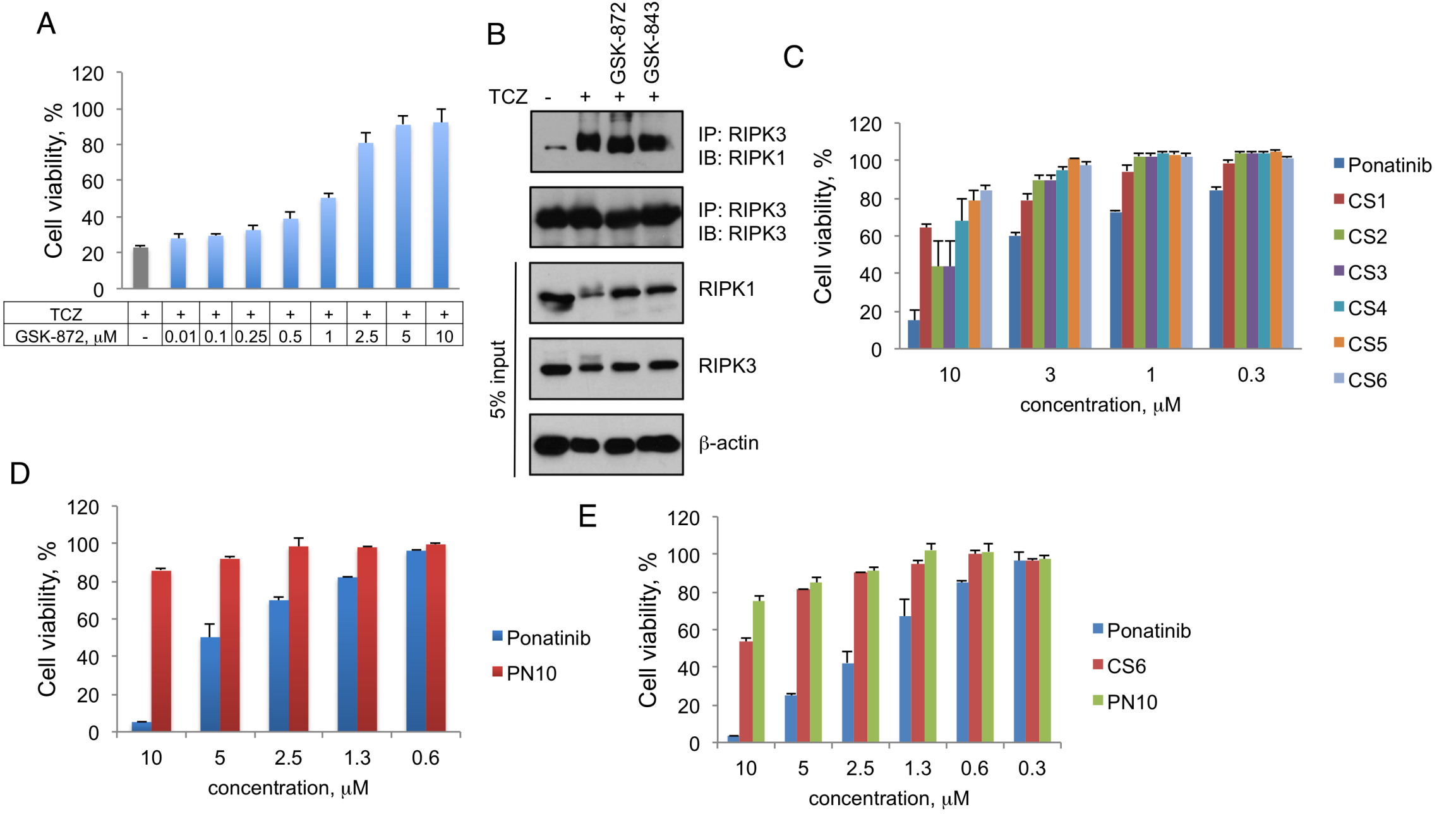

A
Cell viability, %
120
100
80
60
40
20
0
TCZ + + + + + + + + +
GSK-872, μM - 0.01 0.1 0.25 0.5 1 2.5 5 10
B
TCZ - + + GSK-872 + GSK-843
IP: RIPK3
IB: RIPK1
IP: RIPK3
IB: RIPK3
5% input
RIPK1
RIPK3
β-actin
C
Cell viability, %
120
100
80
60
40
20
0
10 3 1 0.3
concentration, μM
Ponatinib
CS1
CS2
CS3
CS4
CS5
CS6
D
Cell viability, %
120
100
80
60
40
20
0
10 5 2.5 1.3 0.6
concentration, μM
Ponatinib
PN10
E
Cell viability, %
120
100
80
60
40
20
0
10 5 2.5 1.3 0.6 0.3
concentration, μM
Ponatinib
CS6
PN10

Figure S5

Supplemental figure legends

Figure S1. Inhibition of necroptosis and RIPK1 kinase by ponatinib, related to Figure 1.

A) Close alignment of Glu-in/DXG-out structures of RIPK1 (blue PDB: 4NEU) and Abl (green, PDB: 3OXZ). Structure alignment was performed using Maestro software package (Schrodinger). B) Screening of a panel of Type 2 inhibitors shows inhibition of necroptosis by ponatinib and DCC-2036. FADD-deficient Jurkat cells were treated with 10 ng/ml human TNFα in the presence of indicated concentrations of inhibitors for 24 hr. Cell viability was determined using CellTiter-Glo assay and normalized relative to non-TNF-treated control. C) Ponatinib, but not Gleevec inhibited RIPK1 kinase *in vitro* in HTRF assay. HTRF KinEASE assay was performed for recombinant RIPK1 kinase using 11-point dose response series for each inhibitor. Inhibition relative to DMSO control was calculated and used to determine IC_{50} values. D) Ponatinib is a more potent inhibitor of necroptosis compared to Nec-1. Necroptosis in immortalized bone marrow macrophage cells was induced for 24 hr by 10 ng/ml LPS and 50 μM zVAD.fmk. Cells were treated with 8 (ponatinib) or 12 (Nec-1) concentrations of each inhibitor. Values of cell viability, relative to non-LPS-treated controls, were determined using CellTiter-Glo assay and used to calculate EC_{50} values. Cell viability data are presented as mean ± SD.

Figure S2. Docked model of ponatinib/RIPK1 complex, related to Figure 2.

A) Close alignment of RIPK2/ponatinib crystal structure (red/brown) and RIPK1/ponatinib docked model (cyan/blue), visualized using Maestro software. B-D) Interactions of ponatinib with Abl (B), RIPK2 (C) and RIPK1 (D). Interaction diagrams were generated using Maestro software package using ponatinib/Abl (PDB: 3OXZ) and ponatinib/RIPK2 (PDB: 4C8B) crystal structures. Ponatinib/RIPK1 docked model was generated as described in Methods section. In all cases, hydrogen bonds to the hinge Met residue, backbone of DXG motif and side-chain of Glu residue of αC helix are seen.

Figure S3. Unfavorable interactions of RIPK1 DFG mutants with CS6 and inhibition of RIPK3-dependent cell death by CS analogs and RIPK3 inhibitor GSK-872, related to Figure 3.

A) MM-GBSA energy profile analysis reveals unfavorable interactions of CS6 with DFG mutants of RIPK1 and T315I mutant of Abl. Energy changes between free and bound sates of ponatinib, CS6 and residues in the Abl and RIPK1 binding pockets were calculated as described in Methods section. Colors indicate energy changes upon small molecule binding from favorable (blue) to unfavorable (red). Side chains of gatekeeper and DXG residues are shown. B-C) GSK-872, but not Abl inhibitors Gleevec and Nilotinib inhibit cell death induced by IFNγ. RIPK1-/- MEFs were treated with 10 ng/ml IFNγ in the presence of indicated concentrations of GSK-872 or 25 μM Nec-1 for 24 hr. Cell viability was determined using CellTiter-Glo assay. Cell viability data are presented as mean ± SD.

Figure S4. Development of hybrid PN RIPK1 inhibitors, related to Figure 4.

A) Glu-out conformation of DLG-out pocket of RIPK1 is substantially larger than that of Glu-in/DLG-out pocket. DLG-out pockets of Glu-out/DLG-out RIPK1/Nec-1 structure (red, PDB: 4ITH) and Glu-in/DLG-out RIPK1/ponatinib docked model (green) are shown. B) Unfavorable contacts between hydantoin of Nec-1 and Met67 residue in Glu-in/DLG-out model of RIPK1. Alignment of Nec-1 (green)/RIPK1 (Glu-out/DLG-out, red, PDB: 4ITH) structure and Glu-in/DLG-out RIPK1 model (blue) was performed using Maestro software. Unfavorable contacts are indicated by red dotted lines. C) Alignment of ponatinib and Nec-1 molecules. Alignment of the binding poses of ponatinib (yellow, from Fig. 2) in RIPK1/ponatinib docked model and Nec-1 in RIPK1/Nec-1 crystal structure (green, PDB: 4ITH) is shown. Methyl of hydantoin of Nec-1 occupies a similar position to carbonyl of central amide of ponatinib, providing a convenient point for attaching ATP pocket fragment of ponatinib. Distance between hydantoin methyl group

of Nec-1 (green) and Ring A of ponatinib (blue) is shown. D) Overall strategy used for the design of PN hybrids. Only candidate inhibitors that are capable of assuming a conformation providing hydrogen bonds in the DLG-out and ATP pockets of Glu-out/DLG-out RIPK1 were selected ("constrained" docking). Resulting molecules were docked without restraints and molecules retaining correct H-bonding were selected for synthesis. E) Alignment of Nec-1 fragment of PN10 from "un-constrained" GLIDE XP docked model (blue) with Nec-1 in RIPK1 X-ray structure (brown, PDB: 4ITH) and ATP pocket fragments of PN10 (blue) and ponatinib (green). Overall, similar, but not identical binding poses were observed. Linker connecting ponatinib and Nec-1 moieties is highlighted in purple. G) Docking scores for different PN molecules were calculated using GLIDE XP.

Figure S5. Cytoprotective properties of ponatinib analogs, related to Figure 5.

A) Inhibition of TCZ-induced necroptosis in MEFs by RIPK3 inhibitor GSK872. Cells were treated with 50 ng/ml mouse TNFα 200 ng/ml cycloheximide, and 25 μM zVAD.fmk for 18 hr in the presence of indicated concentrations of inhibitor, followed by CellTiter-Glo viability assay. B) Necrosome assembly was not affected by two distinct RIPK3 inhibitors, GSK-843 and GSK-872. Cells were treated with 50 ng/ml mouse TNFα, 200 ng/ml cycloheximide, and 25 μM zVAD.fmk for 6 hr in the presence of the inhibitors, followed by RIPK3 immunopricipitation. GSK-843/872 were used at 3 μM. C,E) Reduced cytotoxicity of CS analogs and PN10 in Jurkat cells and MEFs. FADD-deficient Jurkat cells (C,D) and MEFs (E) were treated with indicated concentrations of inhibitors for 24 hr, followed by CellTiter-Glo viability assay. Cell viability data are presented as mean ± SD.

Supplemental tables

Table S1. Full data of ScanEDGE panel screen of CS and PN inhibitors, related to Figures 3 and 4.

Table S2. Inhibition of DFG and non-DFG kinases by Ponatinib and CS6 (from ScanEDGE screening data).

Supplemental Experimental Procedures

Homology modeling

A composite homology model for RIPK1 in the DLG-out/aC-in (Glu-in) form was generated using Prime (Schrödinger LLC) (Farid et al., 2006, Sherman et al., 2006). Based on a BLAST search the crystal structures of LCK kinase (2OFV, 32% sequence identity) and ponatinib-bound RIPK2 (4C8B, 32% sequence homology) were used as templates along with the native structures of RIPK1 (4ITH, 4ITJ and 4ITI). The binding sites of these kinases were aligned (RMSD=1.3Å) and the residue positions corresponding to the aC-helix, DXG-motif and the b-strands of the ATP N-lobe bearing Lys45 catalytic residue were used from the LCK and ponatinib-bound RIPK2 structures. The core sections are defined by the average of Cα atom positions in these regions, and side chain torsion angles are then predicted by simultaneous global optimization of the energy for all non-identical residues. The remainders of the residues positions were used from the native RIPK1 structures. Side-chain rotamers and loops were refined using Prime after the initial model was built. Extended loop sampling was carried out for the activation loop, while the remaining loops were sampled using the standard hieratical sampling protocol in Prime. The Prime generated model was subjected to 2000 cycles of energy minimization using Desmond (DE Shaw research) (Piana et al., 2012). The final model had no steric clashes and >85% of the residues were in the Ramachandran allowed region. A second model of ponatinib bound to DLG-out/Glu-in conformation of RIPK1 was built using the pdb coordinates from the RIPK1 crystal structure (4NEU) and

binding site information from the RIPK2•ponatinib complex (4C8B). Qualitatively, no differences between final model and the one previously built were noted.

Docking calculations and MM-GBSA

Docking calculations were carried out using GLIDE XP (Schrödinger LLC). MM-GBSA calculations were carried out using Prime. Both programs were invoked from the Schrödinger Maestro 9.4 molecular modeling interface. The protein structures were prepared for docking using the pre-wizard workflow. Briefly, hydrogen atoms were added, loops and missing atoms were added wherever necessary. Side chain pK_a estimation was carried out using PropKa (Kieseritzky and Knapp, 2008) and the structures were minimized using OPLS2005 force-field (Lupyan et al., 2012) prior to use in docking. Ligands were prepared for docking using the ligprep workflow and pK_a and tautomers were generated using Epik (Manchester et al., 2010, Shelley et al., 2007). Only the structures with the lowest Epik state penalties were used in the final docking. GLIDE grids were calculated around the ligand with H-bond constrains placed on the backbone amide of Met95 (ponatinib hinge), backbone amide of Leu157 (DLG) and backbone carbonyl of Val97 (Nec-1 binding). Docking calculations were carried out in the extra precision mode. The details of GLIDE XP are described in details in (Friesner et al., 2004). Two set of docking calculations were carried out on all ligand of the PN series 1) With all three H-bond constraints and 2) with no constraints. The strain estimation python script was used to estimate the residual strain on the ligand after docking. Briefly, the script carries out a restrained gas phase minimization of the ligand and the bound conformation of the ligand and estimates total energy in each case. The differences in the two measured energy are used as an approximate estimate of the ligand strain (smaller value equals less strain).

MM-GBSA calculations were carried out with docked models of CS6 in WT RIPK1, mutants of RIPK1 and Abl. A hierarchal sampling protocol within Prime MM-

GBSA with VSGB solvent model was used to explore the flexibility of the DLG motif and the gatekeeper Met (RIPK1) and Thr (Abl) in presence of various ligands. The energy per atom was calculated, stored during this calculation and used to color code individual atoms in the structures.

Recombinant protein expression

Baculoviruses were generated using co-transfection of pACGHTL transfer vectors with linearized Bright BaculoGold baculoviral plasmid (BD Biosciences) using calcium phosphate Sf9 transfection kit (BD Biosciences) as previously described (Maki et al., 2013). GST-RIPK1 was expressed and purified as described (Maki et al., 2012). GST-RIPK3 protein was expressed by co-infection of GST-RIPK3 and Cdc37 baculoviruses in ESF921 Protein Free medium (Expression Systems). After five days of infection, cells were collected and re-suspended in lysis buffer (40 mM HEPES pH 7.3, 150 mM NaCl, 10 mM sodium pyrophosphate, 17.5 mM b-glycerolphosphate, 1 mg/ml aprotonin, 1 mg/ml leupeptin, 1 mg/ml pepstatin, and 50 mg/ml phenylmethanesulfonyl fluoride (PMSF)). Cells were sonicated followed by centrifugation. The resulting lysate was loaded onto a 5-ml Glutathione 4B Sepharose column (GE Healthcare) and eluted with 50 mM glutathione. GST-RIPK3 fractions were combined, concentrated to 1 ml, and injected onto a Superdex 200 10/300 GL column (GE Healthcare) and eluted using an isocratic gradient (50 mM Tris pH 8.0, 150 mM NaCl, and 2 mM b-mercaptoethanol). GST-RIPK3 fractions were combined and concentrated. Glycerol (20%) was added to the protein prior to flash freezing and storage at -80C. A NanoDrop 2000 Spectrophotomer (Thermo Scientific) was used to determine the final protein concentration.

Human His-RIPK2 kinase domain (aa 8-317) was provided by Dr. Alex Bullock (Oxford University) and is described elsewhere (Canning et al., manuscript in

preparation). Recombinant GST-Abl kinase domain protein was purchased from SignalChem.

Radiometric assay

All radiometric kinase assays were performed as previously described either using recombinant kinases or FLAG-RIPK1 expressed and immunopricipitated from HEK293T cells (Degterev et al., 2008). Inhibitors were used at concentrations indicated in the figures with a final DMSO concentration of 3%.

Enzymatic assays

GST-RIPK1 protein was used in the *in vitro* homogeneous time-resolved fluorescence (HTRF) KinEASE Sub 3 assay (Cisbio) as previously described (Maki and Degterev, 2013). For ADPGlo (Promega) assays 1 ng of GST-Abl, 10 ng of His-RIPK2 and 20 ng of GST-RIPK1 and GST-RIPK1 were used. Reactions were performed in 50 mM HEPES, pH 7.5, 50 mM NaCl, 30 mM $MgCl_2$, 1 mM DTT, 0.05% bovine serum albumin (BSA), 0.02% CHAPS (RIPK1/RIPK3), 40 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 0.5 mM DTT, 0.01% BSA (RIPK2) and 40 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 50 mM DTT, 0.1% BSA (Abl). Reactions were supplemented with 50 mM ATP and 1 mg of Abltide (SignalChem, for Abl kinase). Reactions were performed at room temperature for 1 hr (Abl, RIPK2) or 4 hr (RIPK1, RIPK3). Reactions were performed in 5 mL (5% final concentration of DMSO) and stopped by addition of 5 mL of ADPGlo reagent for 40 min at room temperature. Luminescent signal was generated by addition of 10 mL of kinase detection reagent for 30 min at room temperature and determined using Victor3V platereader (Perkin Elmer). Specific signal was calculated by subtracting values in the wells without protein and inhibitor from the values in the test wells. Inhibition, % = ((specific signal (DMSO control) - specific signal (inhibitor))/ (specific signal (DMSO control))) x 100%. Non-linear regression to determine IC_{50} values was performed using Prism software (GraphPad).

Cell viability assays

Cell viability assays in human TNFα-treated FADD-deficient Jurkat cells were performed as previously described (Degterev et al., 2008). Briefly, cells were seeded at $5x10^4$ cells per well in 100 μL of media in white 96 well plates (Costar). Cells were treated with inhibitors in DMSO (0.5% final DMSO concentration) and 10 ng/ml human TNFα (Peprotech) for 24 hr. Luminescent signal was developed by addition of CellTiter-Glo reagent (Promega) and detected using Victor3V platereader. Specific viability relative to non-TNF-treated/inhibitor–treated wells was calculated. Non-linear regression to determine EC_{50} values was performed using Prism software (GraphPad). For activation of cell death by TNFα and 5(z)-7-oxozaenol, MEF cells were seeded at $1x10^4$ cells per well and treated with inhibitors and 10 ng/ml mouse TNFα (Peprotech), 100 nM 5(z)-7-oxozeaenol (Cayman Chemical) and 50 μM zVAD.fmk (Apex Bio) for 24 hr, followed by CellTiter-Glo assay. For activation of cell death by poly(I:C), MEF cells were seeded at $1x10^4$ cells per well and treated with inhibitors and 5 μg/ml mouse TNFα (Peprotech) and 50 μM zVAD.fmk (Apex Bio) for 24 hr, followed by CellTiter-Glo assay. For activation of LPS-dependent necroptosis in iBMM cells, cells were seeded at $2x10^4$ cells per well and treated with inhibitors and 100 ng/ml LPS (0111:B4, Sigma) and 50 μM zVAD.fmk (Apex Bio) for 24 hr, followed by CellTiter-Glo assay. For activation of RIPK3-dependent cell death by IFNγ, *ripk1 -/-* MEFs ($1x10^5$/well) were seeded into six-well plate and treated with 10 ng/ml mouse IFNγ (R&D systems) for 48 hours in the presence of inhibitors, and viability was determined by trypan blue exclusion analysis. For activation of cell death by TCZ, WT MEFs cells were seeded at $1.25x10^5$ cells per well in a 6 well plate and treated with inhibitors and 50 ng/ml mouse TNFα (R&D systems), 250 ng/ml cycloheximide (MP Biomedicals), and 50 μM zVAD.fmk (Bachem) for 24 hr, followed by trypan blue exclusion analysis. For measuring cytotoxicity of ponatinib analogs, FADD-

deficient Jurkat and MEF cells were treated with inhibitors for 24 hr in 6 or 96 well plates, followed by CellTiter-Glo assay.

qRT-PCR

qPCR analysis of changes in human TNFα mRNA was performed as described in (McNamara et al., 2013). Briefly, cells were seeded into 24 well plates at $5X10^5$ cells/well in 1 ml of media. Cells were stimulated with 10 ng/ml hTNFα for 8 hr. For iBMM experiments, cells were seeded in 12 well plates at $5X10^5$ cells/well in 1 ml of media. Cells were stimulated with 10 ng/ml LPS (Sigma, X) and 50 μM zVAD.fmk (Apex Bio) for 7 hr. Total RNA was isolated using ZR-Miniprep kit (Zymo Research), cDNA was synthesized using random primers using iScript cDNA synthesis kit (Bio-Rad) and qPCR reactions were performed using SYBR Green mix (Affymetrix) and gene-specific primers. Sequences of the primers were: hTNF forward – 5'-ATGAGCACTGAAAGCATGATCC-3'; hTNF reverse – 5' GAGGGCTGATTAGAGAGAGGTC-3'; hGAPDH forward – 5'-GAACGGGAAGCTTGTCATCA-3'; hGAPDH reverse 5'-GGTTCAGACCCATGACGAAC-3'; mTNF forward 5'-CCCTCACACTCAGATCATCTTCT-3'; mTNF reverse 5'-GCTACGACGTGGGCTACAG-3'; mGAPDH forward 5'-TGTGTCCGTCGTGGATCTGA-3'; mGAPDH reverse 5'- GGTCCTCAGTGTAGCCCAAG-3'.

Resulting Ct values were used to calculate changes in mRNA levels relative to non-TNFα-treated controls. GAPDH was used as a house keeping control to normalize TNFα mRNA levels.

Western blot

For western blots, equal volumes of samples used in kinase reactions were subjected to SDS-PAGE. Antibody incubations were performed using TBST blocking buffer (Fisher Scientific) and ECL signal was developed using Luminata ECL reagent (Millipore).

RIPK1/RIPK3 necrosome co-IP assay

$5x10^5$ MEFs were seeded into 60 mm plates one day before treatment. Cells were pre-treated with kinase inhibitors at the indicated concentrations (or 3 μM each GSK RIPK3 inhibitor) for 1 hr, and then treated with the combination of 50 ng/ml mouse TNFα (R&D systems), 200 ng/ml cycloheximide (MP Biomedicals), and 25 μM zVAD.fmk (Bachem) for 6 hr. Following treatment, cells were lysed in a lysis buffer (1% Triton X-100, 150mM sodium chloride, 20mM HEPES, 5mM EDTA, 5mM sodium fluoride, 0.2 mM sodium ortho-vanadate supplemented with Complete Mini Protease Inhibitor cocktail (Roche)) and briefly sonicated. Lysates were cleared by centrifugation and incubated overnight with 2 μg of anti-RIPK3 antibody (ProSci) at 4°C with rotation. 30 μL of protein A/G-agarose slurry (Thermo Scientific) was added to lysates and incubated for additional 2 hr. in 4°C with rotation. Immunoprecipitates were washed three times with cold lysis buffer and eluted by directly boiling in SDS sample buffer. Eluted proteins were subjected to 8% SDS-PAGE and Western blotting was performed with anti-RIPK1 antibody (BD Transduction).

Synthesis of inhibitors

Optimized Nec-1, Nec-3 and Nec-4, GSK-843 and GSK-872 were synthesized as previously described (Jagtap et al., 2007, Teng et al., 2005, Teng et al., 2008, Kaiser et al., 2013). Ponatinib, Gleevec, DCC-2036 and type 2 Abl inhibitors were purchased from Selleck Biosciences. Synthesis of CS and PN analogs is described in *Supplemental data*.

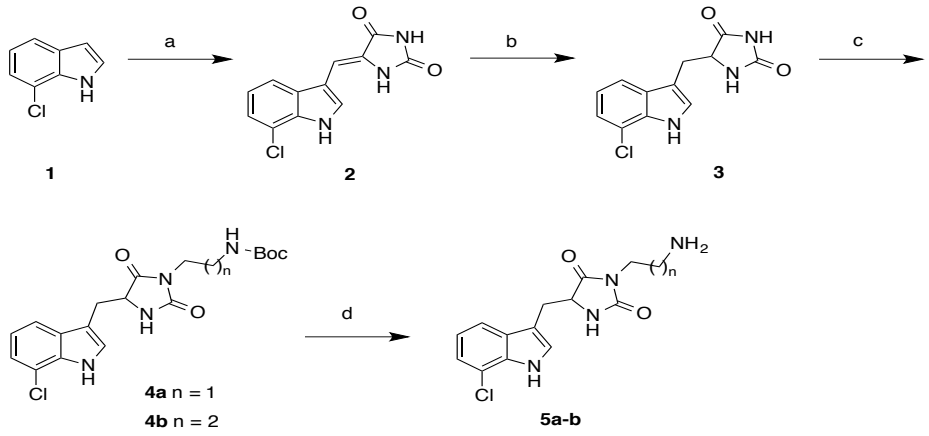
DNA plasmids

The human GST-RIPK1 kinase domain (a.a. 1-327) baculovirus construct was described previously (Maki et al., 2012). Human RIPK3 kinase domain (a.a. 1-313) was constructed into the same pAcGHLT A baculovirus transfer vector (BD Biosciences).

Human RIPK1 kinase cDNA fragment corresponding to a.a. 1-327 was sub-cloned into pcDNA3.1 plasmid. Site-directed mutagenesis was performed using QuikChange II kit (Agilent Technologies).

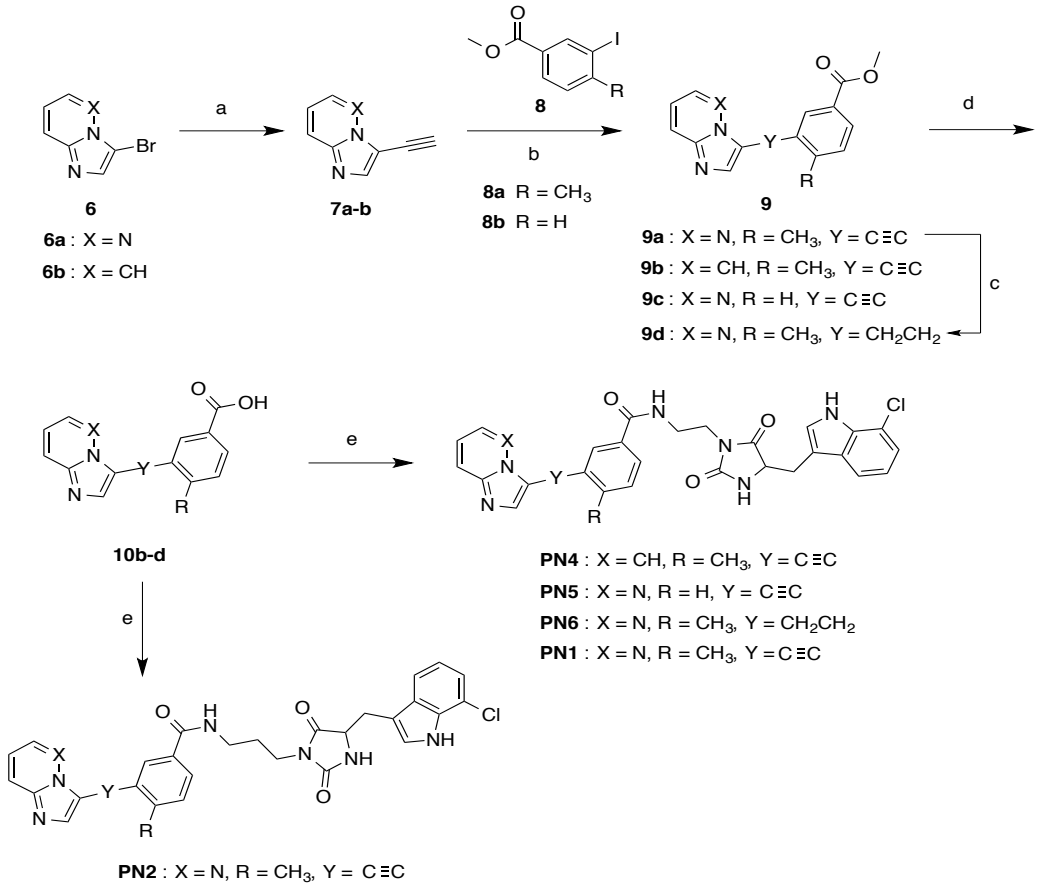
Synthesis of CS and PN analogues

Scheme 1. Synthesis of intermediate **3** and **5a–b**[a]



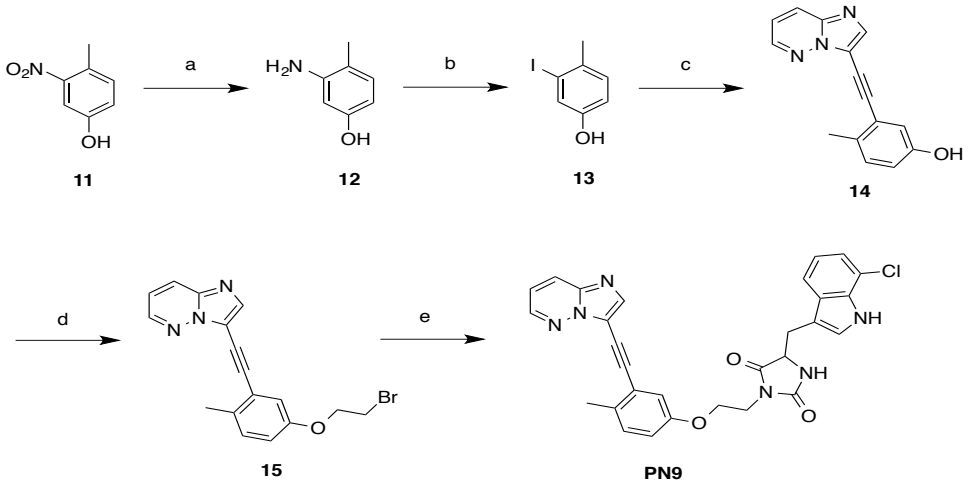
[a] Reagents and conditions: (a) i) $P(O)Cl_3$, DMF, 0 °C, 2 h; ii) hydantoin, 110 °C, overnight; (b) $CoCl_2$, MeOH, THF, $NaBH_4$, 1 h; (c) (*tert*-butoxy)-*N*-(2-bromoethyl)carboxamide or (*tert*-butoxy)-*N*-(2-bromopropyl)carboxamide, NaOEt, EtOH, reflux, 6 h; (d) TFA, rt, 3 h.

Scheme 2. Synthesis of inhibitors **PN1**, **PN2** and **PN4 – PN6**[a]



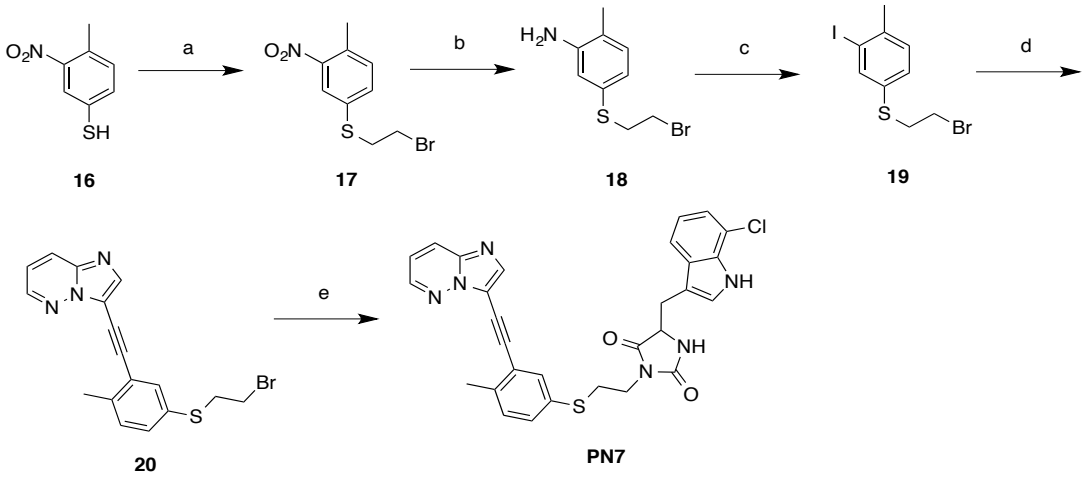
[a] Reagents and conditions: (a) i) ethynyltrimethylsilane, 5 mol% $Pd(PPh_3)_4$, 10 mol% CuI, $(i\text{-Pr})_2NEt$, DMF, rt, 1 h; ii) TBAF, THF, rt, 15 min; (b) 5 mol% $Pd(PPh_3)_4$, 15 mol% CuI, $(i\text{-Pr})_2NEt$, DMF, 80 °C, 2h; (c) 10% Pd/C, H_2, MeOH, rt, 4 h; (d) LiOH, MeOH, rt, 5 h; (e) **5a** or **5b**, HOBt, EDCI, $(i\text{-Pr})_2NEt$, DMF, rt, overnight.

Scheme 3. Synthesis of inhibitors **PN9**[a]



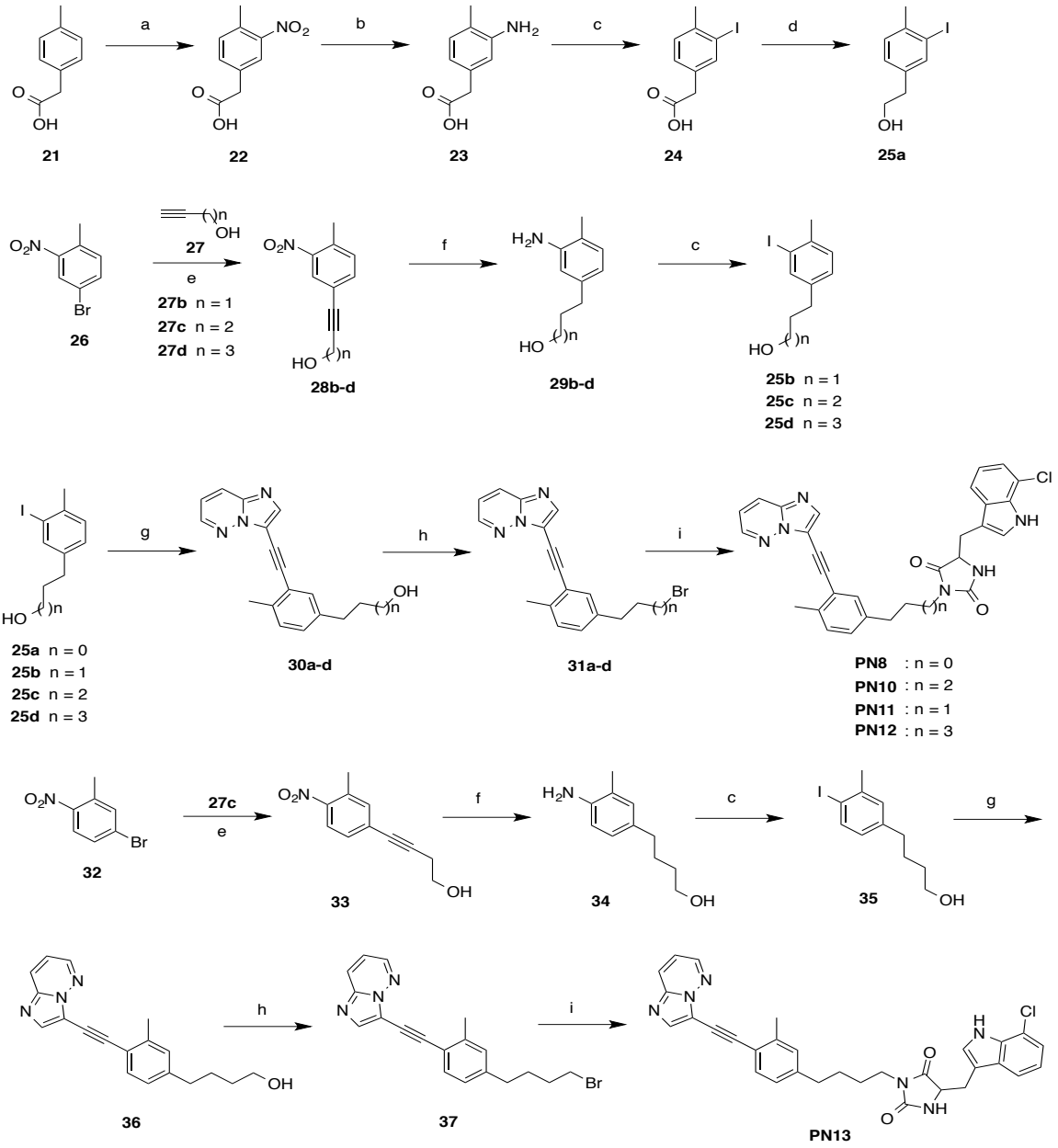
[a] Reagents and conditions: (a) 10% Pd/C, H_2, MeOH, 50 °C, overnight; (b) $NaNO_2$, H_2O, 0 °C, 1 h; ii) KI, rt, overnight; (c) **7a**, 5 mol% $Pd(PPh_3)_4$, 10 mol% CuI, (*i*-Pr)$_2$NEt, DMF, 80 °C, 2 h; (d) 1,2-dibromoethane, NaH, THF, rt, overnight; (e) **3**, NaOEt, EtOH, reflux, 6 h.

Scheme 4. Synthesis of inhibitors **PN7**[a]



[a] Reagents and conditions: (a) 1,2-dibromoethane, K_2CO_3, CH_3CN, 65 °C, overnight; (b) Fe, EtOH, H_2O, 85 °C, 3 h; (c) i) $NaNO_2$, H_2O, 0 °C, 1 h; ii) KI, rt, overnight; (d) **7a**, 5 mol% $Pd(PPh_3)_4$, 10 mol% CuI, (*i*-Pr)$_2$NEt, DMF, 80 °C, 2 h; (e) **3**, NaOEt, EtOH, reflux, 6 h.

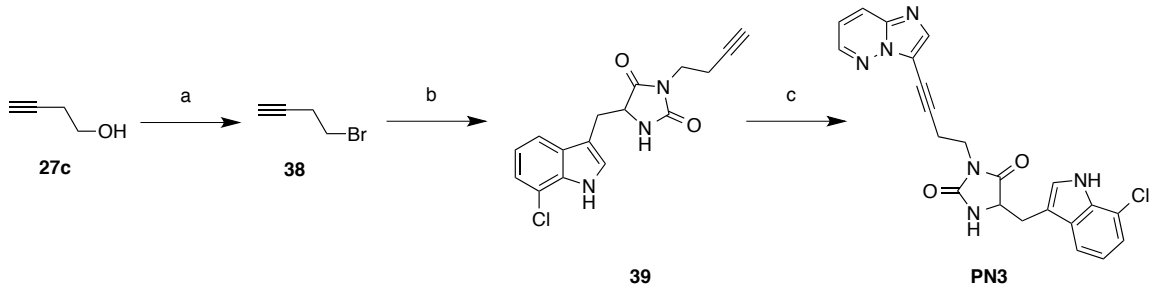
Scheme 5. Synthesis of inhibitors **PN8** and **PN10 – PN13**[a]



[a] Reagents and conditions: (a) HNO_3, CH_2Cl_2, rt, 2 h; (b) 10% Pd/C, MeOH, 50 °C, overnight; (c) i) $NaNO_2$, H_2O, 0 °C, 1 h; ii) KI, rt, overnight; (d) BH_3, THF, reflux, overnight; (e) **27b** or **27c** or **27d**, 5 mol% $Pd(PPh_3)_4$, 10 mol% CuI, $(i\text{-}Pr)_2NEt$, DMF, 80 °C, 2 h; (f) 10% Pd/C, H_2, MeOH, rt, overnight; (g) **7a**, 10 mol% $Pd(PPh_3)_4$, 20 mol%

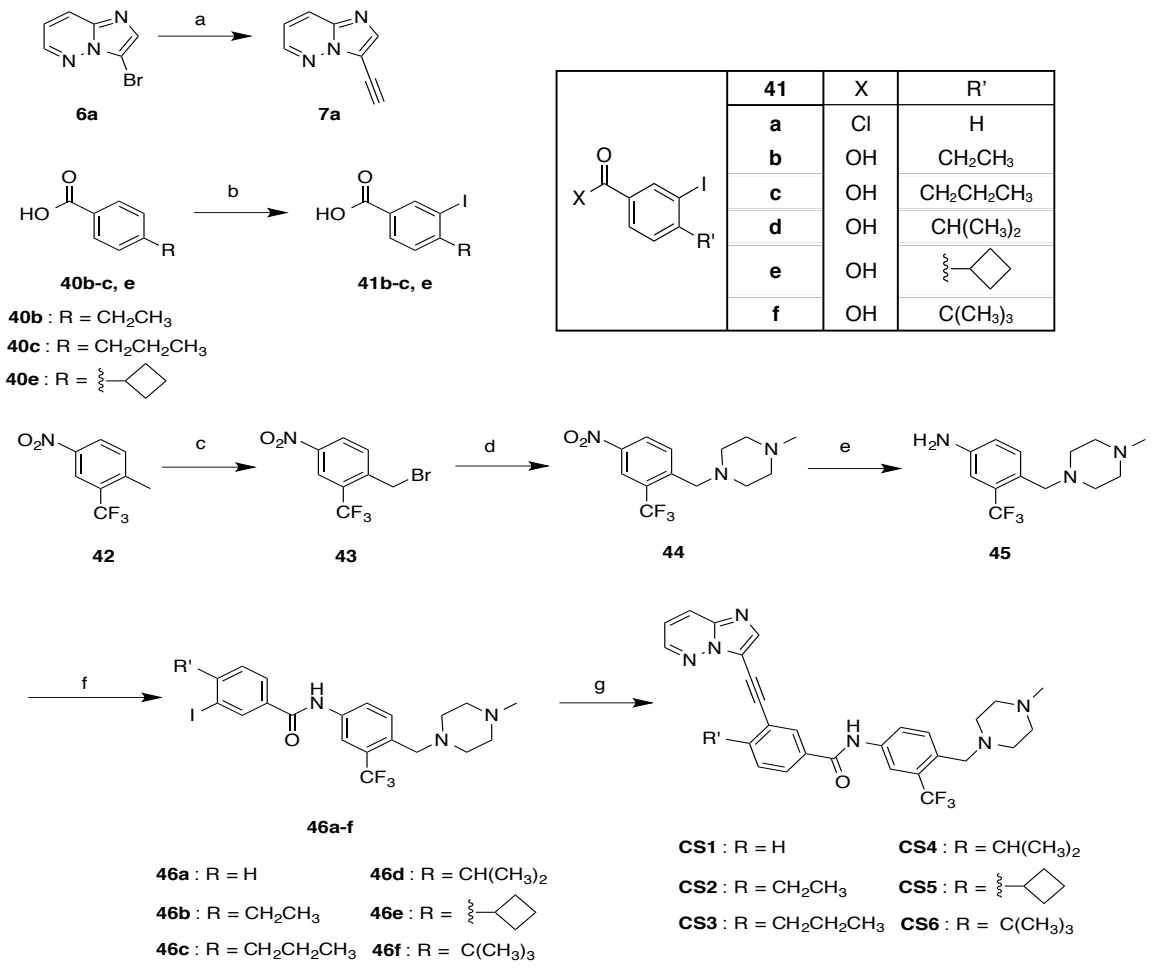
CuI, (*i*-Pr)$_2$NEt, DMF, 80 °C; (h) PBr_3, CH_2Cl_2, rt, overnight; (i) **3**, NaOEt, EtOH, reflux, 6 h.

Scheme 6. Synthesis of inhibitors **PN3**[a]



[a] Reagents and conditions: (a) PBr_3, CH_2Cl_2, rt, overnight; (b) **3**, NaOEt, EtOH, reflux, 6 h; (c) **7a**, 5 mol% Pd(PPh$_3$)$_4$, 10 mol% CuI, (*i*-Pr)$_2$NEt, DMF, 80 °C, 2 h.

Scheme 7. Synthesis of inhibitors **CS1 – CS6**[a]



41	X	R'
a	Cl	H
b	OH	CH_2CH_3
c	OH	$CH_2CH_2CH_3$
d	OH	$CH(CH_3)_2$
e	OH	
f	OH	$C(CH_3)_3$

[a] Reagents and conditions: (a) i) ethynyltrimethylsilane, 10 mol% $Pd(PPh_3)_2Cl_2$, 10 mol% CuI, Et_3N, MeCN, 100 °C, overnight; ii) K_2CO_3, MeOH, rt, 30 min; (b) AgOTf, I_2, CH_2Cl_2, rt, 48 h; (c) *N*-Bromosuccinimide (NBS), 2,2'-azobis(2-methylpropionitrile) (AIBN), 1,2-dichloroethane, reflux, overnight; (d) 1-methylpiperazine, K_2CO_3, CH_2Cl_2, rt, 3 h; (e) 10% Pd/C, H_2, MeOH, rt, 16 h; (f) **41a**, DMAP, $(i\text{-Pr})_2NEt$, THF, rt, 2 h or **41b-f**, HATU, $(i\text{-Pr})_2NEt$, DMF, rt, 16 h; (g) **7a**, 10 mol% $Pd(PPh_3)_2Cl_2$, 10 mol% CuI, Et_3N, reflux, overnight.

General Chemistry Information

All commercially available reagents and solvents were used without further purification except when otherwise stated. All reactions involving air-sensitive reagents were conducted under an argon or nitrogen atmosphere. Thin-layer chromatography (TLC)

was carried out using Baker-flex® silica gel plates (IB2-F). Plates were viewed under UV-light (254 and 365 nm). Flash chromatography was performed on Teledyne ISCO CombiFalsh® R_f. The column was silica gel (230-400 mesh). Compounds **PN1 – PN13** were purified using preparative HPLC and pure fractions were isolated (yields < 10%). The preparative HPLC was done on Waters XB-C18, 30 x 150 mm. Acetonitrile: 0.1% NH_4CO_3 in water: 20~60% was used as a mobile phase at a flow rate of 30 mL/min. ^1H and ^{13}C NMR spectra were recorded at room temperature using Bruker Fourier 300 MHz, Varian Mercury plus 300 MHz or JEOL ECA-500 with tetramethylsilane (TMS) as an internal standard. High-resolution mass spectra (HRMS) were carried by the Department of Chemistry, University of Connecticut.

5-[(7-Chloroindol-3-yl)methylene]-1,3-diazolidine-2,4-dione (2). Phosphoryl chloride (8.43 g, 55 mmol) was added drop-wise to DMF (80 mL) at 0 °C. Compound **1** (7.55 g, 50 mmol) in DMF (16 mL) was added drop-wise. The solution was stirred for 2 h at rt, then anhydrous Na_2CO_3 (20.50 g, 195 mmol) was added at 0 °C with stirring. Hydantoin (5.50 g, 55 mmol) was added and then the temperature was raised to 110 °C and stirred overnight. The reaction was allowed to cool to rt and concentrated. Water and 10% $HCl_{(aq)}$ was added to the residue, and the solid was filtrated and washed with EtOH and water to afford **2** (8.00 g, 61%).

5-[(7-Chloroindol-3-yl)methyl]-1,3-diazolidine-2,4-dione (3). To **2** (1.75 g, 6.5 mmol), $CoCl_2$ (1.70 g, 13 mmol) in MeOH (330 mL), THF (330 mL) at 0 °C was added drop-wise $NaBH_4$ (4.85 g, 130 mmol, in 30 mL EtOH). After 1 hour, conc. HCl was added until no more bubbling and the solution was partially concentrated. Na_2CO_3 aqueous saturated solution was added until pH~7. The mixture was extracted with EtOAc. The organic phase was concentrated to a crude, which was purified by silica gel (5% MeOH in EtOAc) to afford **3** (0.06 g, 35%).

General Procedure for the Synthesis of *tert*-Butyl (3-{4-[(7-chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}alkyl)carbamate 4: *tert*-butyl (2-{4-[(7-chloro-1*H*-indol-3-yl)methyl)]-2,5-dioxoimidazolidin-1-yl}ethyl)carbamate (4a). Compound **3** (1.00 g, 4.4 mmol) was added to NaOEt (4.4 mmol) in 50 mL of anhydrous EtOH. The first mixture was heated to reflux and (*tert*-butoxy)-*N*-(2-bromoethyl)carboxamide (0.70 g, 4.4 mmol) was added. The mixture was heated for 6 h and then diluted with H_2O. The solid was collected and purified by flash column chromatography to afford **4a** (0.06 g, 33%).

***tert*-Butyl (3-{4-[(7-chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}propyl) carbamate (4b).** This compound was made from **3** and (*tert*-butoxy)-*N*-(2-bromopropyl) carboxamide in 38% yield.

General Procedure for the Synthesis of 3-(2-Aminoalkyl)-5-[(7-chloroindol-3-yl)methyl]-1,3-diazolidine-2,4-dione 5: 3-(2-aminoethyl)-5-[(7-chloroindol-3-yl)methyl]-1,3-diazolidine-2,4-dione (5a). A mixture of **4a** (0.60 g, 1.5 mmol) and 10 mL of TFA was stirred at room temperature for 3 h. The excess TFA was removed under reduced pressure and the crude **5a** (0.70 g) was used without further purification.

3-(2-Aminopropyl)-5-[(7-chloroindol-3-yl)methyl]-1,3-diazolidine-2,4-dione (5b). This compound was made from **4b**. The crude product was used directly in next step without further purification.

General Procedure for the Synthesis of Arylacetylenes 7: 3-ethynyl-4-hydroimidazo[1,2-*b*]pyridazine (7a). To a solution of **6a** (3.96 g, 20 mmol) in DMF (15 mL) was added ethynyltrimethylsilane (2.35 g, 24 mmol), $Pd(PPh_3)_4$ (1.15 g, 1 mmol), CuI (0.53 g, 2.8 mmol), and diisopropylethylamine (3.48 mL, 30 mmol). The solution was stirred at rt under N_2 atmosphere for 1 h. The reaction mixture was concentrated and the crude product was purified by silica gel flash chromatography (0-5% MeOH in CH_2Cl_2) to provide 2.80 g of solid. To a solution of the solid (2.80 g, 13 mmol) in THF (20 mL) was

added 15 mL of tetrabutylammonium fluoride (1.0 M in THF) at rt. The solution was stirred for 15 min, concentrated, and the crude product purified by silica gel flash chromatography (0-5 % MeOH in CH_2Cl_2) to provide **7a** (2.02 g, 71%).

3-Ethynyl-4-hydroimidazo[1,2-*a*]pyridine (7b). This compound was made from coupling **6b** and ethynyltrimethylsilane in 74% yield.

General Procedure for the Synthesis of Arylacetylene benzoate 9: methyl 3-(2-(4-hydroimidazo[1,2-*b*]pyridazin-3-yl)ethynyl)-4-methylbenzoate (9a). To a solution of **7a** (429 mg, 3.0 mmol) in DMF (2 mL) was added **8a** (994 mg, 3.6 mmol), $Pd(PPh_3)_4$ (170 mg, 0.15 mmol), CuI (57 mg, 0.3 mmol) and diisopropylethylamine (774 mg, 6 mmol). The reaction mixture was stirred at 80 °C for 2 h under N_2 atmosphere. The reaction mixture was concentrated and the crude product was purified by silica gel chromatography (0-10% MeOH in CH_2Cl_2) to afford **8** (700 mg, 80%).

Methyl 3-(2-(4-hydroimidazo[1,2-*a*]pyridin-3-yl)ethynyl)-4-methylbenzoate (9b). This compound was made from coupling of **7b** and **8a** in 82% yield.

Methyl 3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)benzoate (9c). This compound was made from coupling of **7a** and **8b** in 84% yield.

Methyl 3-(2-(imidazo[1,2-*b*]pyridazin-3-yl)ethyl)-4-methylbenzoate (9d). To a solution of **9a** (582 mg, 2 mmol) in MeOH (50 mL) was added 10% Pd/C (58 mg). The reaction mixture was stirred under 1 atm H_2 at rt for 4 h. The catalyst was filtered off. The crude **9d** (590 mg) was used directly in next step without further purification.

General Procedure for the Synthesis of Arylacetylene benzoic acid 10: 3-(2-(4-hydroimidazo[1,2-*b*]pyridazin-3-yl)ethynyl)-4-methylbenzoic acid (10a). A mixture of **9a** (501 mg, 1.72 mmol), LiOH (124 mg, 5.15 mmol) in 10 mL of MeOH was stirred at rt for 5 h. The pH was adjusted to 5 and the reaction mixture was concentrated and the crude product **10a** (540 mg) was used directly to next step.

3-(2-(4-Hydroimidazo[1,2-*a*]pyridin-3-yl)ethynyl)-4-methylbenzoic acid (10b). This compound was made from **9b**. The crude product **10b** (550 mg) was used without further purification.

3-(2-(4-Hydroimidazo[1,2-*b*]pyridazin-3-yl)ethynyl)benzoic acid (10c). This compound was made from **9c**. The crude product **10c** (540 mg) was used without further purification.

3-(2-(4-Hydroimidazo[1,2-*b*]pyridazin-3-yl)ethyl)-4-methylbenzoic acid (10d). This compound was made from **9d**. The crude product **10d** (540 mg) was used without further purification.

General Procedure for the Synthesis of Inhibitors PN1, **PN2** and **PN4 – PN6: *N*-(3-{4-[(7-chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}propyl)-3-(imidazo[1,2-*b*] pyridazin-3-ylethynyl)-4-methylbenzamide (PN2).** To a solution of **10a** (551 mg, 1.99 mmol) in DMF (10 mL) was added **5b** (639 mg, 1.99 mmol), HOBt (270 mg, 2.0 mmol), EDCI (575 mg, 3.0 mmol) and diisopropylethylamine (1 mL). The reaction mixture was stirred overnight at rt. The mixture was diluted with water and extracted with EtOAc. The combined organic solvents was dried over anhydrous Na_2SO_4, concentrated and purified by prep-HPLC to afford **PN2**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.25-1.50 (m, 2H), 2.56 (s, 3H), 2.96-3.33 (m, 6H), 4.36-4.37 (m, 1H), 6.96 (t, J = 12 Hz, 1H), 7.09-7.13 (m, 1H), 7.19 (d, J = 0.9 Hz, 1H), 7.34-7.52 (m, 3H), 7.78 (dd, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 8.01 (s, 1H), 8.22-8.38 (m, 4H), 8.72 (d, J = 3.0 Hz, 1H), 11.26 (br, 1H). HRMS *m*/*z* calculated for $C_{31}H_{26}ClN_7O_3$ $[M + H]^+$: 580.1859; found: 580.1884.

***N*-(2-{4-[(7-Chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}ethyl)-3-(imidazo[1,2-*a*]pyridin-3-ylethynyl)-4-methylbenzamide (PN4).** This compound was made from coupling of **10b** and **5a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.56 (s, 3H), 3.01-3.104 (m, 2H), 3.20-3.46 (m, 4H), 4.31 (t, J = 5.2 Hz, 1H), 6.94 (t, J = 7.8 Hz, 1H), 7.10-

7.19 (m, 3H), 7.43-7.48 (m, 3H), 7.73 (t, *J* = 8.2 Hz, 1H), 8.03 (s, 2H), 8.20 (s, 1H), 8.56-8.60 (m, 2H), 11.29 (br, 1H). HRMS *m*/*z* calculated for $C_{31}H_{25}ClN_6O_3$ $[M + H]^+$: 565.1755; found: 565.1749.

***N*-(2-{4-[(7-Chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}ethyl)-3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)benzamide (PN5).** This compound was made from coupling of **10c** and **5a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.06-3.10 (m, 2H), 3.23-3.41 (m, 4H), 4.31 (t, *J* = 8.1 Hz, 1H), 6.95 (t, *J* = 7.2 Hz, 1H), 7.13 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 2.1 Hz, 1H), 7.39 (dd, J_1 = 9.2 Hz, J_2 = 4.5 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 8.01 (s, 1H), 8.19-8.20 (m, 2H), 8.26 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H), 8.68-8.70 (m, 2H), 11.27 (br, 1H). HRMS *m*/*z* calculated for $C_{29}H_{22}ClN_7O_3$ $[M + H]^+$: 552.1551; found: 552.1561.

***N*-(2-{4-[(7-Chloro-1H-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}ethyl)-3-(2-(imidazo[1,2-*b*]pyridazin-3-yl)ethyl)-4-methylbenzamide (PN6).** This compound was made from coupling of **10d** and **5a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.30 (s, 3H), 2.97-3.05 (m, 4H), 3.10-3.24 (m, 6H), 4.27-4.31 (m, 1H), 6.89-6.95 (m, 1H), 7.06-7.23 (m, 4H), 7.45 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 5.1 Hz, 1H), 7.62-7.65 (m, 2H), 8.09 (d, *J* = 8.7 Hz, 1H), 8.15 (s, 1H), 8.36-8.40 (m, 1H), 8.56 (d, *J* = 3Hz, 1H), 11.25 (br, 1H). HRMS *m*/*z* calculated for $C_{30}H_{28}ClN_7O_3$
$[M + H]^+$: 570.2020; found: 570.2049.

***N*-(2-{4-[(7-Chloro-1*H*-indol-3-yl)methyl]-2,5-dioxoimidazolidin-1-yl}ethyl)-3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylbenzamide (PN1).** This compound was made from coupling of **10a** and **5a**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H), 3.05-3.10 (m, 2H), 3.24-3.38 (m, 4H), 4.29-4.32 (m, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 7.37-7.49 (m, 3H), 7.75 (dd, J_1 = 8.2 Hz, J_2 = 1.5 Hz, 1H), 7.99 (d, *J* = 1.8 Hz, 1H), 8.18-8.28 (m, 3H), 8.59-8.63 (m, 1H), 8.71 (d, *J* =

4.8 Hz, 1H), 11.27 (br, 1H). HRMS *m*/*z* calculated for $C_{30}H_{24}ClN_7O_3$ $[M + H]^+$: 566.1707; found: 566.1738.

3-Amino-4-methylphenol (12). To a solution of **11** (3.04 g, 20 mmol) in MeOH (50 mL) was added 10% Pd/C (304 mg). The reaction mixture was stirred under 50 psi H_2 at 50 °C overnight. The catalyst was removed by filtration. The crude product **12** (2.26 g) was used directly in next step without further purification.

3-Iodo-4-methylphenol (13). To a solution of **12** (1.11 g, 9 mmol) in H_2O (10 mL) was added 32% H_2SO_4 (18 g). Then the mixture was cooled to 0 °C. Next, $NaNO_2$ (625 mg, 9 mmol) in H_2O (5 mL) was added drop-wise. The reaction mixture was stirred at 0 °C for 1 h then KI (2.2 g, 14 mmol) was added. The mixture was stirred at rt overnight. The mixture was extracted with Et_2O, washed with brine. The combined organic solvents was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography on silica gel (0-10% EtOAc in ether) to afford **13** (1.2 g, 57%) as a brown solid.

3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenol (14). To a solution of **7a** (286 mg, 2 mmol) in DMF (8 mL) was added **13** (562 mg, 2.4 mmol), $Pd(PPh_3)_4$ (113 mg, 0.1 mmol), CuI (38 mg, 0.2 mmol) and diisopropylethylamine (516 mg, 4 mmol). The reaction mixture was stirred at 80 °C for 2 h under N_2 atmosphere. The mixture was concentrated and the crude product was purified by silica gel chromatography (0-10% MeOH in CH_2Cl_2) to afford **14** (370 mg, 74%).

3-{[5-(2-Bromoethoxy)-2-methylphenyl]ethynyl}imidazo[1,2-*b*]pyridazine (15). A mixture of **14** (370 mg, 1.49 mmol) and 60% NaH (71 mg, 1.78 mmol) in THF (20 mL) was stirred at 0 °C for 1 h then 1,2-dibromoethane (335 mg, 1.78 mmol) was added. The reaction mixture was stirred at rt overnight. Water was added and the aqueous phase was extracted with EtOAc. The combined organic phase was washed with brine,

dried over Na_2SO_4 and evaporated. The crude was purified by silica gel chromatography (0-10% MeOH in CH_2Cl_2) to provide **15** (410 mg, 77%).

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-(2-(3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenoxy)ethyl)imidazolidine-2,4-dione (PN9). To NaOEt (68 mg, 1 mmol) in anhydrous EtOH (15 mL) was added **3** (264 mg, 1 mmol). The reaction mixture was heated to reflux and **15** (356 mg, 1 mmol) was added. The mixture was heated for 6 h then evaporated to dryness. The crude was purified by prep-HPLC to afford **PN9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H), 3.04 (dd, J_1 = 14.6 Hz, J_2 = 7.8 Hz, 1H), 3.38 (dd, J_1 = 14.8 Hz, J_2 = 3.9 Hz, 1H), 3.82-3.86 (m, 2H), 3.96-4.00 (m, 2H), 4.31 (dd, J_1 = 8.4 Hz, J_2 = 3.9 Hz, 1H), 5.49 (s, 1H), 6.76 (dd, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 1H), 7.03-7.20 (m, 6H), 7.49 (d, J = 7.8 Hz, 1H), 8.01-8.06 (m, 2H), 8.27 (br, 1H), 8.44 (d, J = 4.2 Hz, J_2 = 7.8 Hz, 1H). HRMS *m*/*z* calculated for $C_{29}H_{23}ClN_6O_3$ $[M + H]^+$: 539.1598; found: 539.1597.

4-(2-Bromoethylthio)-1-methyl-2-nitrobenzene (17). To a solution of **16** (6.0 g, 35.4 mmol) in MeCN (120 mL) was added K_2CO_3 (12.0 g, 88.8 mmol), 1,2-dibromoethane (16.7 g, 88.8 mmol). The reaction mixture was stirred at 65 °C overnight. The mixture was cooled to rt and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography (0-10% EtOAc in ether) to provide **17** (6.1 g, 62%).

5-(2-Bromoethylthio)-2-methylphenylamine (18). A mixture of **17** (5.4 g, 19.2 mmol), Fe (3.3g, 58.2 mmol) in EtOH (300 mL) and H_2O (300 mL) was stirred at 85 °C for 3 h. The mixture was cooled to rt and extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography (0-10% EtOAc in ether) to provide **18** (2.4 g, 51%).

4-(2-Bromoethylthio)-2-iodo-1-methylbenzene (19). This compound was prepared using procedure for **13** in 34% yield.

3-{[5-(2-Bromoethylthio)-2-methylphenyl]ethynyl}imidazo[1,2-*b*]pyridazine (20). This compound was prepared using procedure for **14** in 49% yield.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-[2-(3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenylthio)ethyl]imidazolidine-2,4-dione (PN7). This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.53 (3H, s), 2.97-3.02 (m, 3H), 3.36 (dd, J_1 = 15.9 Hz, J_2 = 2.1 Hz, 1H), 3.67-3.71 (m, 2H), 4.18-4.21 (m, 1H), 5.55 (br, 1H), 7.03-7.12 (m, 3H), 7.19 (td, J_1 = 7.5 Hz, J_2 = 0.6 Hz, 2H), 7.31 (dd, J_1 = 8.1 Hz, J_2 = 2.1 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 2.1 Hz, 1H), 7.98 (dd, J_1 = 9.2 Hz, J_2 = 1.8 Hz, 1H), 8.02 (s, 1H), 8.34 (br, 1H), 8.35 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H). HRMS *m*/*z* calculated for $C_{29}H_{23}ClN_6O_2S$ $[M + H]^+$: 555.1370; found: 555.1311.

2-(4-Methyl-3-nitrophenyl)acetic acid (22). To a solution of **21** (3.0 g, 20.0 mmol) in dried CH_2Cl_2 was added fuming HNO_3 (3 mL) drop-wise. The mixture was stirred at rt for 2 h, then quenched with water and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to provide **22** (3.1 g, 79%).

2-(3-Amino-4-methylphenyl)acetic acid (23). This compound was prepared using procedure for **12**. The crude product (1.63 g) was used directly in the next step without further purification.

2-(3-Iodo-4-methylphenyl)acetic acid (24). This compound was prepared using procedure for **13** in 48% yield.

2-(3-Iodo-4-methylphenyl)ethan-1-ol (25a). A mixture of **24** (1.0 g, 3.6 mmol) and BH_3 (1 M in THF, 36 mL) in 50 mL THF was refluxed overnight. The mixture was evaporated and dissolved in EtOAc, washed with brine, driver over anhydrous Na_2SO_4

and evaporated. The crude was purified by column chromatography on silica gel (0-10% EtOAc in ether) to afford **28a** (710 mg, 75%).

3-(4-Methyl-3-nitrophenyl)prop-2-yn-1-ol (28b). This compound was prepared using procedure for **14** by coupling of **26** and **27b** in 64% yield.

4-(4-Methyl-3-nitrophenyl)but-3-yn-1-ol (28c). This compound was prepared using procedure for **14** by coupling of **26** and **27c** in 38% yield.

5-(4-Methyl-3-nitrophenyl)pent-4-yn-1-ol (28d). This compound was prepared using procedure for **14** by coupling of **26** and **27d** in 48% yield.

General Procedure for the Synthesis of 5-(3-Amino-4-methylphenyl)alkan-1-ol 29: 3-(3-amino-4-methylphenyl)propan-1-ol (29b). To a solution of **28b** (2.8 g, 14.7 mmol) in MeOH (100 mL) was added 10% Pd/C (280 mg). The reaction mixture was stirred under 50 psi H_2 at rt overnight. The catalyst was removed by filtration. The crude product (2.3 g) was used directly in next step without further purification.

4-(3-Amino-4-methylphenyl)butan-1-ol (29c). This compound was made from reduction of **28c**. The crude product (1.3 g) was used without further purification.

5-(3-Amino-4-methylphenyl)pentan-1-ol (29d). This compound was made from reduction of **28d**. The crude product (2.0 g) was used without further purification.

3-(3-Iodo-4-methylphenyl)propan-1-ol (25b). This compound was prepared using procedure for **13** in 22% yield.

4-(3-Iodo-4-methylphenyl)butan-1-ol (25c). This compound was prepared using procedure for **13** in 21% yield.

5-(3-Iodo-4-methylphenyl)pentan-1-ol (25d). This compound was prepared using procedure for **13** in 29% yield.

General Procedure for the Synthesis of Arylacetylene alkanol 30: 2-(3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl)ethan-1-ol (30a). A mixture of **7a** (286 mg, 2 mmol), **25a** (629 mg, 2.4 mmol), $Pd(PPh_3)_4$ (113 mg, 0.1 mmol), CuI (38

mg, 0.2 mmol) and diisopropylethylamine (516 mg, 4 mmol) in DMF (8 mL) was stirred at 80 °C for 2 h under N_2 atmosphere. The reaction mixture was concentrated and the crude product was purified by silica gel chromatography (0-5% MeOH in CH_2Cl_2) to afford **30a** (349 mg, 63%).

3-(3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl)propan-1-ol (30b). This compound was made from coupling of **7a** and **25b** in 63% yield.

4-(3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl)butan-1-ol (30c). This compound was made from coupling of **7a** and **25c** in 60% yield.

5-(3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl)pentan-1-ol (30d). This compound was made from coupling of **7a** and **25d** in 64% yield.

General Procedure for the Synthesis of 3-{2-[5-(5-Bromoalkyl)-2-methylphenyl]ethynyl}-4-hydroimidazo[1,2-*b*]pyridazine 31: 3-{[5-(2-bromoethyl)-2-methyl phenyl]ethynyl}imidazo[1,2-*b*]pyridazine (31a). To a solution of **30a** (305 mg, 1.0 mmol) in CH_2Cl_2 (8 mL) was added PBr_3 (543 mg, 2.0 mmol). The reaction mixture was stirred at rt overnight under N_2 atmosphere. The mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated. The crude **31a** (400 mg) was used directly in next step without further purification.

3-{[5-(3-Bromopropyl)-2-methylphenyl]ethynyl}imidazo[1,2-*b*]pyridazine (31b). This compound was made from **30b**. The crude product (410 mg) was used without further purification.

3-{[5-(4-Bromobutyl)-2-methylphenyl)ethynyl)imidazo[1,2-*b*]pyridazine (31c). This compound was made from **30c**. The crude product (1.2 g) was used without further purification.

3-{[5-(4-Bromopentyl)-2-methylphenyl)ethynyl)imidazo[1,2-*b*]pyridazine (31d). This compound was made from **30d**. The crude product (440 mg) was used without further purification.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-[3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenethyl]imidazolidine-2,4-dione (PN8). This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.38 (t, J_1 = 7.2 Hz, 2H), 2.46 (s, 3H), 3.04-3.06 (m, 2H), 3.37-3.39 (m, 2H), 4.32-4.35 (m, 1H), 6.88 (dd, J_1 = 8.1 Hz, J_2 = 1.8 Hz, 1H), 6.96 (t, J = 7.8 Hz, 1H), 7.12 (d, J = 6.9 Hz, 1H), 7.14-7.24 (m, 3H), 7.36 (dd, J_1 = 9.0 Hz, J_2 = 4.5 Hz, 1H), 7.49 (d, J = 8.1 Hz, 1H), 8.16 (s, 2H), 8.23 (dd, J_1 = 9.2 Hz, J_2 = 2.1 Hz, 1H), 8.68 (dd, J_1 = 4.2 Hz, J_2 = 1.8 Hz, 1H), 11.27 (br, 1H). HRMS *m*/*z* calculated for $C_{29}H_{23}ClN_6O_2$ $[M + H]^+$: 523.1649; found: 523.1622.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-{3-[3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl]propyl}imidazolidine-2,4-dione (**PN11**)**.** This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.21-1.27 (m, 2H), 2.06-2.10 (m, 2H), 2.45 (s, 3H), 3.01-3.16 (m, 5H), 4.32-4.34 (m, 1H), 6.92-6.98 (m, 2H), 7.07-7.09 (m, 1H), 7.15-7.21 (m, 3H), 7.35 (dd, J_1 = 9.3 Hz, J_2 = 4.5 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 8.16-8.24 (m, 3H), 8.68 (dd, J_1 = 6 Hz, J_2 = 1.5 Hz, 1H), 11.22 (br, 1H). HRMS *m*/*z* calculated for $C_{30}H_{25}ClN_6O_2$
$[M + H]^+$: 537.1806; found: 537.1804.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-{4-[3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl]butyl}imidazolidine-2,4-dione (PN10). This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34-1.43 (m, 4H), 2.48-2.53 (m, 5H), 3.10 (dd, J_1 = 14.8 Hz, J_2 = 7.8 Hz, 1H), 3.32-3.49 (m, 3H), 4.26-4.30 (m, 1H), 5.55 (br, 1H), 7.02-7.20 (m, 6H), 7.33 (dd, J = 1.8 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.98-8.03 (m, 2H), 8.40-8.43 (m, 1H). HRMS *m*/*z* calculated for $C_{31}H_{27}ClN_6O_2$ $[M + H]^+$: 551.1962; found: 551.1963.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-{5-[3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl]pentyl}imidazolidine-2,4-dione (PN12). This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.69-0.77 (m, 2H), 0.93-0.98 (m, 2H), 1.21-1.31 (m, 2H), 2.34 (t, *J* = 8.4 Hz, 2H), 2.45 (s, 3H), 3.05-3.12 (m, 4H), 4.30-4.32 (m, 1H), 6.95 (t, *J* = 7.8 Hz, 1H), 7.08-7.11 (m, 2H), 7.17 (d, *J* = 2.4 Hz, 1H), 7.23 (d, *J* = 8.1 Hz, 1H), 7.30-7.36 (m, 2H), 7.49 (d, *J* = 7.8 Hz, 1H), 8.15 (s, 2H), 8.21 (dd, J_1 = 9.0 Hz, J_2= 1.5 Hz, 1H), 8.67 (dd, J_1 = 4.6 Hz, J_2 = 1.5 Hz, 1H), 11.24 (br, 1H). HRMS *m*/*z* calculated for $C_{32}H_{29}ClN_6O_2$ $[M + H]^+$: 565.2119; found: 565.2112.

4-(3-Methyl-4-nitrophenyl)but-3-yn-1-ol (33). This compound was prepared using procedure for **14** by coupling of **32** and **27c** in 38% yield.

4-(4-Amino-3-methylphenyl)but-3-yn-1-ol (34). This compound was made from reduction of **33** (1.8 g, 8.8 mmol) using procedure for **29b**. The crude product (1.3 g) was used without further purification.

4-(4-Iodo-3-methylphenyl)but-3-yn-1-ol (35). This compound was prepared using procedure for **13** in 21% yield.

4-(3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl)butan-1-ol (36). This compound was prepared using procedure for **30a** by coupling of **35** and **7a** in 60% yield.

3-{[5-(4-Bromobutyl)-2-methylphenyl]ethynyl}imidazo[1,2-*b*]pyridazine (37). This compound was prepared using procedure for **31a** starting from **36**. The crude product (600 mg) was used without further purification.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-{4-[3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-methylphenyl]butyl}imidazolidine-2,4-dione (PN13). This compound was prepared using procedure for **PN9**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.03-1.06 (m, 3H), 1.94-2.02 (m, 1H), 2.25-2.32 (m, 2H), 2.46 (s, 3H), 3.07-3.15 (m, 4H), 4.26-4.30 (m, 1H), 6.93-6.98 (m, 2H), 7.04 (s, 1H), 7.11 (d, *J* = 6.9 Hz, 1H), 7.17 (d, *J* = 2.4 Hz, 1H),

7.34 (dd, J_1 = 9.2 Hz, J_2 = 4.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 8.14 (s, 1H), 8.21 (dd, J_1 = 9.3 Hz, J_2 = 1.5 Hz, 1H), 8.68 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H), 11.24 (br, 1H). HRMS *m*/*z* calculated for $C_{31}H_{27}ClN_6O_2$ $[M + H]^+$: 551.1962; found: 551.1945.

4-Bromobut-1-yne (38). This compound was prepared using procedure for **31a** and the crude product was used directly in next step without further purification.

3-(But-3-yn-1-yl)-5-[(7-chloro-1*H*-indol-3-yl)methyl]imidazolidine-2,4-dione (39). This compound was made from coupling of **3** and **38** using procedure for **PN9**.

5-[(7-Chloro-1*H*-indol-3-yl)methyl]-3-[4-(imidazo[1,2-*b*]pyridazin-3-yl)but-3-yn-1-yl]imidazolidine-2,4-dione (PN3). This compound was synthesized from coupling of **39** and **7a** using procedure for **14**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.44-2.51 (m, 2H), 3.04-3.16 (m, 2H), 3.38-3.49 (m, 2H), 4.39-4.43 (m, 1H), 6.98 (t, J = 8.1 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.21 (d, J = 2.1 Hz, 1H), 7.30-7.32 (m, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.95-8.20 (m, 3H), 8.61 (d, J = 4.2 Hz, 1H), 11.29 (br, 1H). HRMS *m*/*z* calculated for $C_{22}H_{17}ClN_6O_2$ $[M + H]^+$: 433.1180; found: 433.1159.

3-Ethynylimidazo[1,2-*b*]pyridazine (7a). Compound **6a** (300 mg, 1.51 mmol), $Pd(PPh_3)_2Cl_2$ (107 mg, 0.15 mmol), CuI (29 mg, 0.15 mmol) were added to a round-bottom flask then the mixture was flushed with Ar for 10 min. MeCN (10 mL), triethylamine (0.4 mL, 3.03 mmol) and ethynyltrimethylsilane (0.4 mL, 3.03 mmol) were added. The reaction was stirred and refluxed overnight under Ar atmosphere. The catalyst was filtered off and solvent was removed under vacuum. MeOH (10 mL) and K_2CO_3 (419 mg, 3.03 mmol) were added and stirred at rt for 30 min. MeOH was removed under vacuum then it was diluted with CH_2Cl_2 and wash with water and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-50% EtOAc in hexane) to provide a brown solid **7a** (128 mg, 50%).

General Procedure for the Synthesis of 4-Alkyl-3-iodobenzoic acid 41: 4-ethyl-3-iodobenzoic acid (41b). To a solution of **40b** (300 mg, 2.00 mmol) in CH_2Cl_2 (3 mL) was added AgOTf (514 mg, 2.00 mmol) and I_2 (508 mg, 2.00 mmol). The reaction was protected from light and stirred at rt for 48 h. The reaction was filtered through celite pad and diluted with CH_2Cl_2 washed with 5% $NaHS_2O_3$, water and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product **41b** (250 mg) was used directly in next step without further purification.

3-Iodo-4-propylbenzoic acid (41c). This compound was made from iodination of compound **40c** (300 mg, 1.83 mmol) to provide a white solid **41c** (307 mg).

4-Cyclobutyl-3-iodobenzoic acid (41d). This compound was made from iodination of compound **40d** (46 mg, 0.26 mmol) to provide a pale yellow solid **41d** (89 mg).

1-(Bromomethyl)-4-nitro-2-(trifluoromethyl)benzene (43). To a solution of **42** (1.31 g, 6.38 mmol) in 1,2-dichloroethane (15 mL) was added *N*-bromosuccinimide (NBS) (1.36 g, 7.65 mmol) and 2,2'-azobis(2-methylpropionitrile) (AIBN) (0.10 g, 0.64 mmol). The reaction was refluxed overnight. The reaction was diluted with CH_2Cl_2 and washed with water and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-5% EtOAc in hexane) to provide a yellow oil **43** (863 mg, 48%).

1-Methyl-4-(4-nitro-2-(trifluoromethyl)benzyl)piperazine (44). To a solution of **43** (179 mg, 0.63 mmol) in CH_2Cl_2 (2 mL) was added 1-methylpiperazine (0.14 mL, 1.26 mmol) and K_2CO_3 (87 mg, 0.63 mmol). The reaction was stirred at rt for 3 h. The reaction was diluted with CH_2Cl_2 and washed with water and saturated NaCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-30% EtOAc in hexane) to provide a yellow oil **44** (188 mg, 98%).

4-[(4-Methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)aniline (45). To a solution of **44** (155 mg, 0.51 mmol) in MeOH (5 mL) was added 10% Pd/C (16 mg). The reaction flask was purged with H_2 and stirred at rt under H_2 atmosphere overnight. The reaction was filtered through celite pad and concentrated. The crude compound **45** (123 mg) was used directly in next step without further purification.

3-Iodo-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (46a). To a solution of **45** (20 mg, 0.07 mmol) in THF (2 mL) was added **41a** (0.01 mL, 0.06 mmol), diisopropylethylamine (0.02 mL, 0.09 mmol) and catalytic amount of 4-dimethylaminopyridine (DMAP). The reaction was stirred at rt for 2 h. EtOAc was added to diluted the reaction and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-10% MeOH in CH_2Cl_2) to provide a yellow oil **46a** in quantitative yield.

General Procedure for the Synthesis of 3-Iodo-N-(3-(trifluoromethyl)phenyl)-benzamide 46: 4-ethyl-3-iodo-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) phenyl}benzamide (46b). To a solution of **45** (46 mg, 0.17 mmol) in DMF (3 mL) was added **41b** (60 mg, 0.22 mmol), HATU (70 mg, 0.22 mmol) and diisopropylethylamine (0.09 mL, 0.50 mmol). The reaction was stirred at rt overnight. EtOAc was added to dilute the reaction and wash with water and saturated NaCl. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-20% MeOH in CH_2Cl_2) to provide a yellow oil **46b** in 50% yield.

3-Iodo-4-propyl-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl} benzamide (46c). This compound was made from coupling of **45** (50 mg, 0.18 mmol) and **41c** (69 mg, 0.24 mmol) to provide **46c** as a yellow oil in 96% yield.

3-Iodo-4-isopropyl-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) phenyl}benzamide (46d). This compound was made from coupling of **45** (50 mg, 0.18 mmol) and **41d** (64 mg, 0.22 mmol) to provide a yellow oil **46d** in 68% yield.

4-Cyclobutyl-3-iodo-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) phenyl}benzamide (46e). This compound was made from coupling of **45** (30 mg, 0.11 mmol) and **41e** (50 mg, 0.17 mmol) to provide a yellow oil **46e** in 56% yield.

4-(*tert*-Butyl)-3-iodo-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) phenyl}benzamide (46f). This compound was made from coupling of **45** (54 mg, 0.20 mmol) and **41f** (72 mg, 0.24 mmol) to provide a yellow oil **46f** in 79% yield.

General Procedure for the Synthesis of Inhibitors CS1-CS6: 3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl} benzamide (CS1). A solution of **46a** (60 mg, 0.12 mmol), **7a** (26 mg, 0.18 mmol), $Pd(PPh_3)_2Cl_2$ (8 mg, 0.01 mmol), CuI (2 mg, 0.01 mmol) and triethylamine (5 mL) was flushed with Ar for 10 min. The reaction mixture was refluxed overnight. It was allowed to cool to rt and diluted with EtOAc, then washed with water and saturated NaCl. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel flash chromatography (0-20% MeOH in CH_2Cl_2) to provide a yellow solid **CS1** in 62% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.28 (s, 3H), 2.49 (br, 8H), 3.61 (s, 3H), 7.07 (dd, J_1 = 9.2 Hz, J_2 = 4.0 Hz, 1H), 7.42 (t, J = 8.0 Hz, 1H), 7.67 (d, J = 6.9Hz, 1H), 7.73 (d, J = 8.6Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.94-7.97 (m, 2H), 8.03 (s, 1H), 8.08 (s, 1H), 8.44 (dd, J_1 = 3.7 Hz, J_2 = 1.7 Hz, 1H), 9.21 (s, 1H). HRMS *m*/*z* calculated for $C_{28}H_{25}F_3N_6O$ $[M + H]^+$: 519.2120; found: 519.2141.

4-Ethyl-3-(imidazo[1,2-b]pyridazin-3-ylethynyl)-*N*-{4-[(4-methylpiperazin-1-yl) methyl]-3-(trifluoromethyl)phenyl}benzamide (CS2). This compound was made from coupling of **46b** (45 mg, 0.08 mmol) and **7a** (18 mg, 0.13 mmol) to provide a pale yellow solid **CS2** in 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (t, J = 7.4 Hz, 3H), 2.30 (s, 3H), 2.51 (br, 8H), 2.97 (q, J = 7.4 Hz, 2H), 3.63 (s, 2H), 7.01 (dd, J_1 = 9.2 Hz, J_2 = 4.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 7.86 (dd, J_1 = 8.0 Hz, J_2 = 2.3 Hz, 1H), 7.89 (dd, J_1 = 9.4 Hz, J_2 = 1.7 Hz, 1H), 7.94-7.95 (m, 2H), 8.03 (d, J = 1.7 Hz, 1H), 8.10 (s, 1H), 8.46 (dd, J_1 = 4.5 Hz, J_2 = 1.7 Hz, 1H), 8.69 (s, 1H). HRMS *m*/*z* calculated for $C_{30}H_{29}F_3N_6O$ $[M + H]^+$: 547.2433; found: 547.2447.

3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}-4-propylbenzamide (CS3). This compound was made from coupling of **46c** (72 mg, 0.13 mmol) and **7a** (28 mg, 0.20 mmol) to provide a pale yellow solid **CS3** in 81% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.00 (t, J = 7.4 Hz, 3H), 1.74 (sex, J = 7.4 Hz, 2H), 2.29 (s, 3H), 2.50 (br, 8H), 2.90 (t, J = 8.0 Hz, 2H), 3.62 (s, 2H), 7.08 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 9.2 Hz, 1H), 7.84 (dd, J_1 = 8.0 Hz, J_2 = 1.7 Hz, 1H), 7.95-7.96 (m, 2H), 8.02 (d, J = 1.7 Hz, 1H), 8.09 (s, 1H), 8.44 (dd, J_1 = 4.3 Hz, J_2 = 1.7 Hz, 1H), 8.97 (br, 1H). HRMS *m*/*z* calculated for $C_{31}H_{31}F_3N_6O$ $[M + H]^+$: 561.2590; found: 561.2585.

3-(Imidazo[1,2-*b*]pyridazin-3-ylethynyl)-4-isopropyl-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (CS4). This compound was made from coupling of **46d** (67 mg, 0.12 mmol) and **7a** (27 mg, 0.18 mmol) to provide a yellow solid **CS4** in 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (d, J = 6.9 Hz, 6H), 2.30 (s, 3H), 2.52 (br, 8H), 3.62-3.67 (m, 3H), 7.12 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.78 (d, J = 9.2 Hz, 1H), 7.90 (dd, J_1 = 8.0 Hz, J_2 = 2.3 Hz, 1H), 7.92-7.95 (m, 3H), 8.05 (d, J = 2.3 Hz, 1H), 8.09 (s, 1H), 8.41 (s, 1H), 8.48

(dd, J_1 = 4.3 Hz, J_2 = 1.7 Hz, 1H). HRMS *m*/*z* calculated for $C_{31}H_{31}F_3N_6O$ $[M + H]^+$: 561.2590; found: 561.2580.

4-Cyclobutyl-3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (CS5). This compound was made from coupling of **46e** (34 mg, 0.06 mmol) and **7a** (13 mg, 0.09 mmol) to provide a yellow solid **CS5** in 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.90 (m, 1H), 2.07-2.27 (m, 3H), 2.35 (s, 3H), 2.42-2.88 (m, 10H), 3.65 (s, 2H), 4.07 (quin, *J* = 8.6 Hz, 1H), 7.13 (dd, J_1 = 8.9 Hz, J_2 = 4.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.90-7.95 (m, 4H), 8.02 (d, *J* = 1.7 Hz, 1H), 8.08 (s, 1H), 8.42 (s, 1H), 8.48 (d, *J* = 2.9 Hz, 1H). HRMS *m*/*z* calculated for $C_{32}H_{31}F_3N_6O$ $[M + H]^+$: 573.2590; found: 573.2583.

4-(*tert*-Butyl)-3-(imidazo[1,2-*b*]pyridazin-3-ylethynyl)-*N*-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide (CS6). This compound was made from coupling of **46f** (68 mg, 0.12 mmol) and **7a** (26 mg, 0.18 mmol) to provide a yellow solid **CS6** in 41% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.58 (s, 9H), 2.31 (s, 3H), 2.51 (br, 8H), 3.63 (s, 2H), 7.08 (dd, J_1 = 9.2 Hz, J_2 = 4.6 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 8.6 Hz, 1H), 7.94-7.96 (m, 2H), 8.08 (s, 1H), 8.09 (d, *J* = 2.3 Hz, 1H), 8.43 (dd, J_1 = 4.1 Hz, J_2 = 1.2 Hz, 1H), 8.79 (s, 1H). HRMS *m*/*z* calculated for $C_{32}H_{33}F_3N_6O$ $[M + H]^+$: 575.2746; found: 575.2756.

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